THIAMINE METABOLISM OF WOMEN ON CONTROLLED DIETS

I. DAILY URINARY THIAMINE EXCRETION AND ITS RELATION TO CREATININE EXCRETION[1, 2]

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ONE FIGURE

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The determination of urinary thiamine excretion has been used in many laboratories in assessing the thiamine nutrition of man since the picture it gives is fairly reliable. Mickelsen, Caster and Keys ('47) stated that "thiamine excretion values appear in a general way to be linearly related to the thiamine intake." Alexander, Landwehr and Mitchell ('46) reported that "the excretion of thiamine into the urine by normal subjects is directly related to the amount of the vitamin administered."

Adamson et al. ('45) indicated that the excretion of thiamine is related to the excretion of creatinine, and that this ratio is fairly constant from voiding to voiding in a given

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individual. If this is true, the amount of thiamine, in micrograms per gram of creatinine, found in a single voiding of urine would indicate the thiamine nutrition of the individual. The use of this method would mean a great saving of time to the analyst. No further information concerning this method of estimating thiamine nutrition has been found in the literature.

This paper reports data on the daily urinary excretion of thiamine and creatinine of subjects on a controlled intake. The nutritional status of the subjects with respect to thiamine was evaluated on the bases of (1) total daily excretion of thiamine, (2) percentage of thiamine intake excreted, and (3) the ratio of thiamine to creatinine in the urine, expressed as micrograms of thiamine per gram of creatinine.

The per cent excretion of a 5-mg oral test dose of thiamine hydrochloride was determined for some of the subjects. In addition, a very brief study was conducted to assess the validity of estimating the status of thiamine nutrition of an individual from the thiamine to creatinine ratio in single voidings. Determinations were also made on two subjects on controlled diets but ingesting therapeutic amounts of thiamine.

EXPERIMENTAL

This work formed a part of a larger investigation conducted during the years 1949, 1950 and 1951. The purpose of the entire experiment was to investigate the effect of two different levels of thiamine intake on the thiamine nutrition of apparently normal women on a controlled diet, as shown by determinations of urinary excretion of thiamine and creatinine, and the concentration of thiamine in the blood.

Each year, the study was divided into two periods: (1) when the daily thiamine intake was maintained at 500 μg per 1,000 Cal., by the addition of 400 μg of thiamine hydrochloride in aqueous solution to the thiamine provided by the diet, and (2) when the daily thiamine intake was maintained at 300 μg per 1,000 Cal., the amount provided by the diet. The

duration of periods 1 and 2 was, respectively, 31 and 21 days in 1949, 19 and 15 days in 1950 and 15 and 14 days in 1951.

Diet and supplement

The diet used was a modification of the diet of Gifft and Hauck ('46). It provided approximately 70 gm of protein, 80 gm of fat and 260 gm of carbohydrate, to give a total daily intake of approximately 2,000 Cal. Food tables [5] were used to evaluate these constituents. Representative samples were analyzed for thiamine by a modification of the method of Hennessy and Cerecedo ('39), and it was found that approximately 600 μg were provided by this diet. The foods were weighed and the quantity of each consumed daily remained constant throughout the experimental periods. During the 1949 study, the diet was planned to provide only 25 mg of ascorbic acid per day, in order that a special concurrent study could be made of the levels of ascorbic acid in blood and urine (Davey et al., '52). During the 1950 and 1951 studies, the riboflavin content of the diet was maintained at 1.2 mg per day. With these exceptions, the diet was adequate with respect to all other known nutrients.

Subjects

There were 4 subjects for the 1949 study, three subjects for the 1950 study, and 4 subjects for the 1951 study. M.L.W. served as a subject during all three studies and C.A.S. served as a subject during the 1949 and 1951 studies. The subjects were women graduate students and one staff member, and ranged in age from 24 to 44 years. Three of them (M.L.W., H.H.Y. and K.D.) are Chinese and S.W.W. is Korean.

Methods

Urinary thiamine was determined by a modification of the thiochrome method of Hennessy and Cerecedo ('39). The

[5] "Composition of Foods, Raw, Processed, Prepared" by the Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, Agriculture Handbook 8, 1950.

suggestion of Mickelsen, Condiff and Keys ('45) of adjusting the pH just before extracting the thiochrome was followed. Urinary creatinine was determined by Folin's method as described by Hawk, Oser and Summerson ('47).

RESULTS AND DISCUSSION

The first 5 days of each period of controlled thiamine intake were considered as an adjustment time, and the values for urinary thiamine and creatinine for those days were omitted in calculating the data shown in table 1.

The results were evaluated on the following bases suggested as evidences of adequate thiamine nutrition: (1) a mean daily urinary thiamine excretion of 100 μg (Mason and Williams, '42); (2) a mean daily urinary excretion of 13% of the thiamine consumed (Gifft and Hauck, '46; Melnick, Field and Robinson, '39); and (3) a mean daily urinary excretion of 150 μg of thiamine per gram of creatinine excreted (Adamson et al., '45).

(1) On the basis of a mean daily thiamine excretion of 100 μg, an intake of 500 μg per 1,000 Cal. was judged to be adequate for all of the subjects. An intake of 300 μg per 1,000 Cal. was judged to be adequate for only one of the subjects (H.H.Y.). Although M.L.W. excreted over 100 μg per day in period 2, 1949, she did not do so in the two subsequent studies. Therefore, an intake of 300 μg per 1,000 Cal. was judged to be inadequate for her. Variation in day-to-day excretion was greater for all subjects during period 1 (fig. 1).

(2) On the basis of a mean daily urinary thiamine excretion of 13% or more of the intake as a criterion of good nutrition, an intake of 500 μg per 1,000 Cal. was judged to be sufficient for all but one subject (H.A.L.). This subject had an average 24-hour excretion of just 100 μg. An intake of 300 μg per 1,000 Cal. was judged to be adequate for two subjects (B.L.D. and H.H.Y.). Again, M.L.W. excreted more than the critical percentage of her intake in 1949, but not in 1950 and 1951.

(3) On the basis of the urinary excretion of 150 μg of thiamine per gram of creatinine as the critical ratio, the higher thiamine intake was judged to be adequate for all but two subjects (H.A.L. and C.A.S.). The lower thiamine intake was judged to be adequate for none of the subjects. H.A.L. and C.A.S. excreted less than 50 μg of thiamine per gram of creatinine during period 2. According to Adamson et al.

TABLE 1

The urinary excretion of thiamine and creatinine of women on controlled diets

YEAR	SUBJECT	DAILY THIAMINE INTAKE	AVERAGE URINARY EXCRETION			
			Thiamine	Thiamine	Creatinine	Thiamine/ Creatinine
		μg/1,000 Cal.	*μg/24 hr.*	*% of intake*	*gm/24 hr.*	*μg/gm*
1949	M.L.W.	495	227	22.7	1.10	206
		299	107	17.8	1.08	99
	B.L.D.	495	229	22.9	0.83	276
		299	84	14.0	0.80	105
	C.A.S.	495	142	14.2	1.42	100
		299	48	8.0	1.37	35
	H.H.Y.	495	246	24.6	1.05	234
		299	109	18.2	1.02	107
1950	M.L.W.	484	163	16.3	1.07	152
		288	50	8.3	1.07	47
	K.D.	484	184	18.4	1.09	169
		288	57	9.5	1.08	53
	S.W.W.	484	159	15.9	0.84	189
		288	61	10.2	0.89	69
1951	M.L.W.	504	268	26.8	1.15	233
		308	77	12.8	1.11	69
	H.A.L.	504	100	10.0	1.25	80
		308	34	5.7	1.30	26
	C.A.S.	504	133	13.3	1.48	90
		308	47	7.8	1.56	30
	R.B.D.	504	276	27.6	1.10	250
		308	70	11.7	1.09	64
1949	B.W.C.[1,2]	1548	806	27.1	1.05	768
		1339	549	21.4	1.01	544
1950	H.H.Y.[1,3]	2327	1078	17.9	1.07	1007
		2172	1000	17.8	1.08	926

[1] These subjects were ingesting therapeutic amounts of a vitamin preparation containing thiamine.

[2] B.W.C. was under a doctor's supervision for a nephritic condition.

[3] This subject received 2,600 Cal. daily.

DAILY THIAMINE EXCRETION BY ADULTS RECEIVING CONTROLLED DIETS WITH TWO LEVELS OF THIAMINE INTAKE

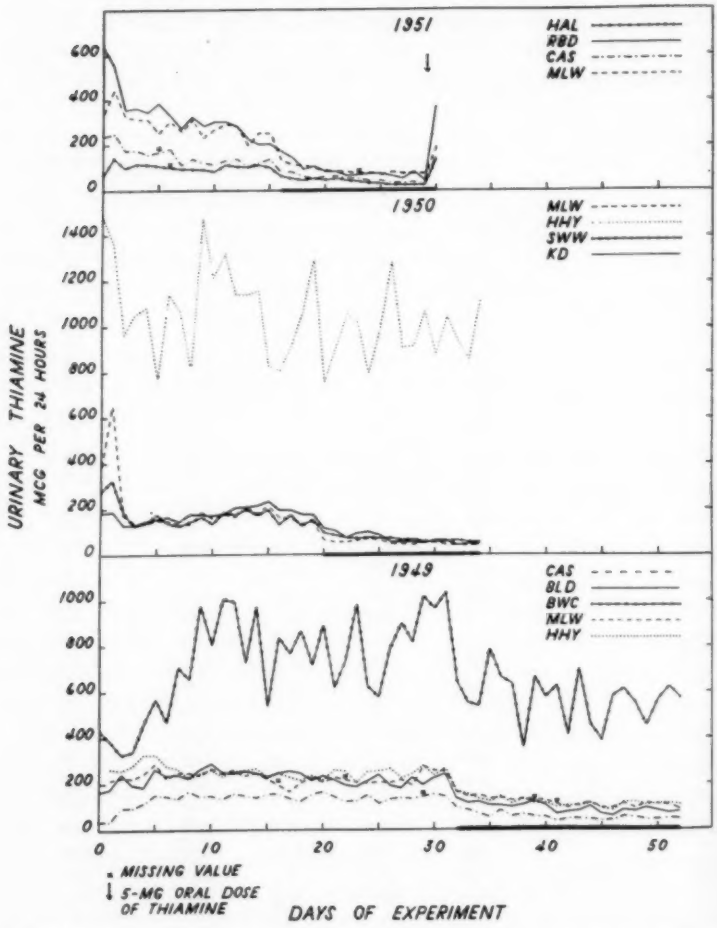

Fig. 1 The periods of lowered thiamine intake are indicated by the heavier base lines.

('45), an excretion of about 50 μg of thiamine per gram of creatinine suggests subnormal thiamine nutrition. Evaluation of thiamine nutritional status by this means gives results which agree generally with those obtained by the other means.

By all three methods of evaluation, it appears that a daily thiamine intake of 500 μg per 1,000 Cal. was sufficient for 6 of the 8 subjects but probably borderline for the other two. This would suggest that the recommended daily allowance of the National Research Council ('48) does not provide a very large safety factor in all instances. A daily intake of 300 μg per 1,000 Cal. appeared, by all three methods of evaluation, to be inadequate for 6 subjects and borderline for the other two.

The fact that there is wide variation in the daily excretion of thiamine when the intake level is high (fig. 1) is not of clinical importance, since even the lower excretions during period 1 would have yielded the same conclusions regarding thiamine nutrition as did the average values. Since the daily fluctuations are much smaller when the excretion is less, the chances of mis-evaluating a thiamine-deficient person on the basis of one day's excretion are also less.

ISOLATED OBSERVATIONS

Five-milligram oral test dose

At the end of the 1951 study, the 4 subjects ingested orally a 5-mg test dose of thiamine hydrochloride, and the 24-hour excretion of the test dose was determined. All of the subjects excreted less than 7% of the dose (M.L.W., 2.4%; H.A.L., 2.1%; C.A.S., 2.2%; and R.B.D., 6.6%) which, according to Melnick, Field and Robinson ('39) and substantiated by Robinson, Melnick and Field ('40) and Gifft and Hauck ('46), is indicative of deficient thiamine stores. This is further evidence that the 300 gm of thiamine per 1,000 Cal. intake of period 2 was not sufficient for these subjects. R.B.D. had taken a liver and vitamin preparation prior to the study, and her excretion of a higher percentage of the test dose was

probably an indication that she had maintained greater thiamine stores than the other subjects.

Ratio of thiamine to creatinine in separate voidings

After the close of the 1951 study, each subject collected and preserved separately each voiding for two days. A summary of the results is given in table 2. It can be seen that there is variation of the thiamine to creatinine ratio in separate voidings, particularly when the ratio was high. However, there were only two of the 34 instances which were very far

TABLE 2

Ratio of thiamine to creatinine in separate voidings for 4 subjects in two 24-hour periods

SUBJECT	RATIO OF THIAMINE TO CREATININE			
	First day		Second day	
	Range in separate voidings	24-hour value	Range in separate voidings	24-hour value
	μg/gm	μg/gm	μg/gm	μg/gm
M.L.W.	276–371	314	367–621	431
H.A.L.	101–108	105	132–157	146
C.A.S.	144–250	184	167–209	180
R.B.D.	183–508	249	149–224	180

from the 24-hour ratios. The use of the urinary thiamine to creatinine ratio in single voidings as a means of evaluation of thiamine nutrition would seem to be warranted, particularly under survey conditions, since this ratio was, for practical purposes, the same as the 24-hour ratio, and the evaluation of thiamine nutrition by using the 24-hour ratio led to the same conclusions as evaluation by other methods.

Values for two subjects who were receiving thiamine supplements

These subjects followed controlled but modified diets. The thiamine intakes and the values found are given in table 1

(B.W.C., 1949, and H.H.Y., 1950). As would be expected, the urinary excretion of thiamine of these subjects was greater than that of the normal subjects throughout periods 1 and 2.

SUMMARY

When 8 normal women were maintained on an intake of 500 μg of thiamine per 1,000 Cal. the average daily urinary excretions of thiamine ranged from 100 to 276 μg. When the thiamine intake was reduced to 300 μg of thiamine per 1,000 Cal. the average daily urinary excretions of thiamine ranged from 34 to 109 μg. The thiamine excretion expressed as per cent of thiamine intake ranged from 10.0 to 27.6 for the higher intake period and from 5.7 to 18.2 for the lower intake period. Urinary thiamine excretions expressed in terms of micrograms of thiamine per gram of urinary creatinine ranged from 80 to 276 for the first period, and from 26 to 107 for the second period of intake.

Using an excretion of 100 μg of thiamine per 24 hours, an excretion of 13% of the daily thiamine intake and an excretion of 150 μg of thiamine per gram of creatinine as indications of good nutrition with respect to thiamine, an intake of 500 μg of thiamine per 1,000 Cal. was judged to be adequate for 6 of the 8 subjects and borderline for the other two. An intake of 300 μg of thiamine per 1,000 Cal was inadequate for 6 of the subjects and borderline for the other two.

The response of 4 subjects to a 5-mg oral test dose of thiamine hydrochloride given on the last day of the period of lowered thiamine intake indicated that the tissues of all the subjects were low in thiamine.

The determination of thiamine in micrograms per gram of creatinine in individual voidings seemed to indicate that the ratio of thiamine to creatinine is fairly constant and, except in two of 34 instances, could have been used as well as the 24-hour ratio for a rough estimation of the status of thiamine nutrition of the subjects.

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THIAMINE METABOLISM OF WOMEN ON CONTROLLED DIETS

II. DAILY BLOOD THIAMINE VALUES [1, 2]

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ONE FIGURE

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No report has been found in the literature in which daily fasting blood thiamine values have been determined for subjects whose thiamine and caloric intakes have been known and controlled for extended periods. It was felt desirable to know how greatly the levels of thiamine in the blood fluctuate under controlled conditions. This paper is a report of the values obtained for thiamine in whole blood and of calculated values of thiamine in packed blood cells. In addition, the experiment was planned to determine whether any change in blood thiamine concentration would occur when the thiamine intake was reduced from 500 μg to 300 μg per 1,000 Cal.

Some determinations of blood thiamine were also made on two subjects on controlled diets but ingesting therapeutic

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[2] This study was made possible by grants from the Williams-Waterman Fund of the Research Corporation of New York City.

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amounts of thiamine, on two ambulatory patients receiving thiamine therapy, and on 5 normal people having self-selected diets.

EXPERIMENTAL

The determination of blood thiamine formed a part of a more extensive investigation conducted during the years 1949, 1950 and 1951. There were 4 subjects in the 1949 and 1951 studies and three in 1950. A detailed description of the subjects, of the diet which afforded approximately 2,000 Cal. daily, and of the plan of the experiment has been given in the report by Louhi, Yü, Hawthorne and Storvick ('52).

Collection of blood samples

Every morning before breakfast, free-flowing blood from a finger puncture was collected in a heparinized [5] 3 × 100 mm capillary tube for hematocrit determination and in a small vial for thiamine determination. Aliquots of blood for the latter were measured immediately so that no anticoagulant was needed.

Method of analysis

Whole blood thiamine was determined by a modification of the microthiochrome method of Burch et al. ('52a). Results were calculated by reference to a standard curve based on the recovery of various amounts of thiamine hydrochloride added to whole blood. Thiamine values were calculated for packed cells from the whole blood values and hematocrit readings, assuming that 10% of the whole blood thiamine is contained in the plasma. This assumption may not hold in every instance. According to some preliminary studies made in this laboratory, the plasma contained between 14 and 15% of the total blood thiamine. However, for the purpose of comparison with Burch et al. ('50) it was assumed that the plasma contained 10% of the total thiamine in the blood.

[5] Heparin was obtained through the courtesy of Roche-Organon, Nutley, New Jersey.

The results of analyses performed during the first 10 days of the 1949 study were not considered valid because of loss of activity in the enzyme preparation. Consequently, the remainder of the samples obtained in 1949, and all of those obtained in 1950, were deproteinized, frozen in dry ice, and stored in a deep freeze until analysis in 1951 when a satisfactory and stable concentrate of acid phosphatase had been prepared. The samples from the 1951 study were analyzed immediately upon collection. The activity of the enzyme preparation was tested using p-nitrophenyl phosphate and cocarboxylase [6] as substrates. It was observed that fluorometric readings of samples which had been frozen were higher than those of aliquots of the same sample analyzed immediately upon collection. For this reason, the samples frozen for one and two years were evaluated using a standard curve based on the recovery of various amounts of thiamine hydrochloride added to whole blood, which was then deproteinized and frozen before being analyzed.

RESULTS

The daily values for micrograms of thiamine per 100 ml of whole blood are shown in figure 1. It can be seen that there were individual differences and considerable variation from day to day for a given individual during either period 1 when the thiamine intake was 500 µg per 1,000 Cal. or period 2 when the intake was 300 µg per 1,000 Cal. It can also be seen that the lowering of the thiamine intake was not reflected in a proportional lowering of the blood thiamine values.

The average of 204 determinations made during all of the periods when the daily thiamine intake was 500 µg/1,000 Cal. (called "periods 1"), was 3.75 µg per 100 ml of whole blood; the average of 193 determinations when the daily thiamine intake was approximately 300 µg/1,000 Cal., made during all of the periods (called "periods 2"), was 3.40 µg. The overall

[6] Cocarboxylase was obtained through the courtesy of Merck and Company, Rahway, New Jersey.

range was from 2.1 to 6.1. The individual average values are given in table 1.

R.B.D., prior to the 1951 study, had taken a liver and vitamin preparation, and her blood thiamine values were

Daily blood thiamine in adults receiving controlled diets with two levels of thiamine intake

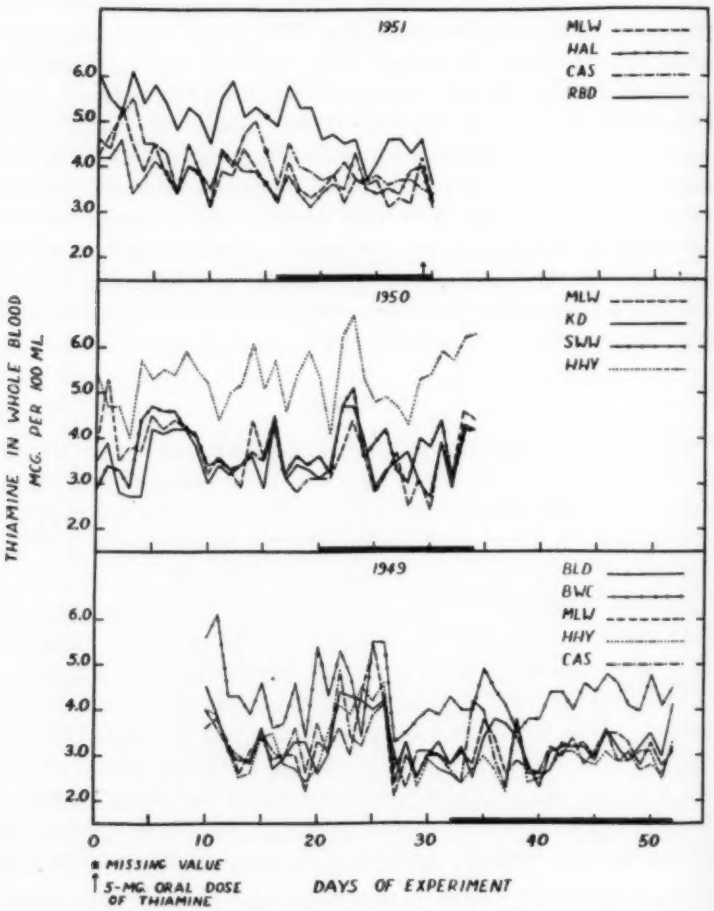

Fig. 1 The periods of lowered thiamine intake are indicated by the heavier base lines.

consistently higher throughout the experimental period (fig. 1 and table 1) although her excretion of thiamine was similar to that of the other subjects (Louhi et al., '52).

Analysis of variance indicated that variation in blood thiamine due to change in thiamine intake was significant in 1951 but not significant in 1949 and 1950.

TABLE 1

Blood thiamine levels of women on controlled diets

YEAR	SUBJECT	DAILY THIAMINE INTAKE	AVERAGE BLOOD THIAMINE LEVEL	
		µg/1,000 Cal.[1]	*µg/100 ml blood*	*µg/100 ml packed cells*
1949	M.L.W.	495	3.3[2]	7.3[2]
		299	3.1	6.8
	B.L.D.	495	3.4[2]	8.6[2]
		299	3.2	7.9
	C.A.S.	495	3.3[2]	7.0[2]
		299	3.0	6.3
	H.H.Y.	495	3.3[2]	8.1[2]
		299	2.8	6.6
1950	M.L.W.	484	3.8	8.2
		288	3.4	7.6
	K. D.	484	3.5	8.1
		288	3.6	8.6
	S.W.W.	484	3.7	8.5
		288	3.9	8.7
1951	M.L.W.	504	4.1	8.9
		308	3.5	7.5
	H.A.L.	504	4.0	9.2
		308	3.6	8.1
	C.A.S.	504	4.4	9.0
		308	3.8	7.6
	R.B.D.	504	5.4	11.2
		308	4.6	10.0
1949	B.W.C.[3]	1548	4.4[2]	9.4[2]
		1339	4.3	9.3
1950	H.H.Y.[3, 4]	2327	5.2	12.0
		2172	5.4	12.5

[1] Daily intake: 2,000 Cal.

[2] Blood thiamine data for the first 10 days were not included.

[3] These subjects were ingesting therapeutic amounts of a vitamin preparation containing thiamine.

[4] This subject received 2,600 Cal. daily.

The average of 188 determinations made during all the "periods 1" was 8.49 μg of thiamine per 100 ml of packed cells, and the average of 186 determinations made during all the "periods 2" was 7.69. The overall range was from 4.7 to 13.0 μg per 100 ml of packed cells. The individual average values are shown in table 1.

Isolated observations

Values for two subjects who were receiving thiamine supplements. Daily fasting blood thiamine determinations were made during the 1949 study for an additional subject, B.W.C., who was under a doctor's supervision for a nephritic condition. She consumed a modified diet, containing approximately 2,000 Cal. per day and a daily intake of 2,970 μg of thiamine in period 1, and 2,570 μg of thiamine in period 2. Values were obtained for an additional subject, H.H.Y., during the 1950 study. She consumed a diet containing 2,600 Cal. per day and a daily intake of 6,000 μg of thiamine in period 1, and 5,600 μg of thiamine in period 2. The values obtained for these subjects are shown in figure 1 and table 1. Their blood thiamine concentrations were higher than those of the other subjects throughout the studies.

Values for two ambulatory patients receiving thiamine therapy. J.P., a woman, was taking an intramuscular injection of a multiple vitamin preparation (containing 10 mg of thiamine hydrochloride) once a week. A blood sample, collected one hour after the injection, gave a thiamine value of 13.4 μg per 100 ml of whole blood. Another sample collected the following week, just before the next injection, gave a value of 6.1. H.H., a man, was taking 50 mg of thiamine by mouth every morning. A blood sample was collected some time before lunch. The whole blood thiamine value was 9.5 μg per 100 ml.

Values obtained from laboratory workers on freely selected diets. At intervals during a period of two months blood samples were secured from 5 laboratory workers who were presumably normal and well-fed. None of them took vitamin

preparations. The results, expressed as micrograms of thiamine per 100 ml of whole blood, were: B.W.C., 12 determinations, average: 4.6, range: 3.8–5.2; R.B.D., 10 determinations, average: 4.5, range: 3.7–5.6; C.A.S., 4 determinations, average: 4.4, range: 3.9–4.9; M.T., 3.6 and 3.5; and E.S., 5.0 and 5.0.

TABLE 2

Response to a 5-mg oral test dose of thiamine hydrochloride by subjects maintained on a daily thiamine intake of 300 µg per 1,000 Cal. for 15 days

SUBJECT	BLOOD THIAMINE		URINARY THIAMINE
	Time after test dose		On day of test
	0	1 hr.	
	µg/100 ml	*µg/100 ml*	*µg/24 hr.*
M.L.W.	4.2	5.1	195
H.A.L.	4.0	3.8	131
C.A.S.	3.5	3.9	142
R.B.D.	4.6	5.6	376

TABLE 3

Response to a 5-mg oral test dose of thiamine hydrochloride by subjects on a freely chosen diet for one month

SUBJECT	BLOOD THIAMINE					URINARY THIAMINE
	Time after test dose					On day of test
	0	½ hr.	1 hr.	2 hr.	3 hr.	
	µg/100 ml					*µg/24 hr.*
M.L.W.	4.7	4.7	5.1	4.4	4.2	366 [1]
H.A.L.	4.0	4.8	4.9	4.7	4.6	331
C.A.S.	4.7	5.0	5.0	4.5	4.8	466
R.B.D.	4.6	5.4	5.6	4.9	4.6	318

[1] One voiding lost, 9 hours after test dose.

Response to a test dose. On the last day of the 1951 study, the 4 subjects were given a 5-mg test dose of thiamine hydrochloride by mouth after a fasting blood sample had been taken. One hour later a second blood sample was collected. According to the urinary data (Louhi et al., '52) all of the subjects were unsaturated. The results are given in table 2.

A month after the close of the 1951 study, another 5-mg test dose of thiamine hydrochloride was administered to the subjects in the fasting state. After taking the test dose solution, each subject had 6 soda crackers and black coffee. Samples of blood were collected just before the administration of the test dose and one-half, one, two and three hours after the test dose. Results are shown in table 3. All of the subjects showed their maximal value after one hour, and in one case the maximum was reached after one-half hour. There was only one case in which there was a significant increase in the response to the test dose.

DISCUSSION

Values for thiamine in whole blood reported in this paper are somewhat lower than values for adults reported by workers using other thiochrome methods. The average of determinations made during all the "periods 1" was 3.75 μg per 100 ml. No subject was in a state of inadequate thiamine nutrition according to the urinary data (Louhi et al., '52). In contrast, Burch et al. ('50) found that in Bataan an average value of 3.7 ± 0.12 μg of thiamine per 100 ml of blood was associated with doubtful cases of beriberi. These workers further found ('50, '52b) that the level of thiamine in the blood of non-symptomatic persons studied in Bataan was 4.0 ± 0.18 μg in 1948 and 4.3 ± 0.09 in 1950, and that the level of thiamine in the blood of well-fed New Yorkers averaged 6.0 μg per 100 ml. Friedemann and Kmieciak ('43) reported a mean non-fasting blood thiamine value for 7 women of 5.60 (3.0–9.2) μg per 100 ml. These were laboratory workers and assumed to be in good nutrition with respect to thiamine. Greenberg and Rinehart ('45) reported a range of blood thiamine values from 5.0 to 10 μg per 100 ml. Their subjects were "normal" laboratory workers and medical students.

However, Chieffi and Kirk ('50) considered whole blood thiamine values ranging from 0 to 2.0 μg per 100 ml to be low

and values ranging from 3.5 to 6.0 to be normal. Our average values are all above their "low" classification.

The correlation coefficient between values in whole blood and values calculated for packed cells was very high ($r = 0.95$ for 112 pairs of observations). This is to be expected as long as the hematocrit values are within normal limits, as was the case in this experiment. However, the admonition of Hennessy ('47) that hematocrit values should be obtained in case there is a large deviation from normal in cell concentration is worthy of consideration, since, as Goodhart and Sinclair ('39) pointed out, most of the vitamin is contained in the cells.

SUMMARY

Eight women served as subjects in studies designed to follow the daily fluctuations of thiamine in whole blood when the thiamine intake was controlled at 500 and 300 μg of thiamine per 1,000 Cal. for periods of two weeks or more. The diet was constant within each study, the only variable being the level of thiamine from one period to the other.

With an intake of about 500 μg of thiamine per 1,000 Cal. the average fasting blood thiamine ranged from 3.3 to 5.4 μg per 100 ml of whole blood or 7.0 to 11.2 μg per 100 ml of packed cells. When the thiamine intake was lowered to 300 μg of thiamine per 1,000 Cal. the average fasting blood thiamine ranged from 2.8 to 4.6 μg per 100 ml of whole blood or 6.3 to 10.0 μg per 100 ml of packed cells.

The effect of a 5-mg oral test dose of thiamine hydrochloride on the level of thiamine in the blood is presented.

A few values for blood thiamine obtained for people other than experimental subjects are included.

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THE PANTOTHENIC ACID CONTENT OF THE BLOOD AND MILK OF SWINE FED SUPPLEMENTAL LEVELS OF THE VITAMIN[1]

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Since the discovery of the importance of pantothenic acid in swine nutrition by Hughes ('42), increasing interest has been shown in levels of this vitamin in various feedstuffs as well as in body tissues and fluids. A limited number of studies of the pantothenic acid content of the blood and milk of swine have been reported in recent years. Luecke et al. ('50) found that blood levels of pantothenic acid in swine seemed to be related to the level of this vitamin which was ingested. Davis et al. ('51) reported the results of pantothenic acid assays of sows' milk which indicated that levels of the vitamin increased as lactation progressed.

The results to be reported in this paper were obtained during the course of a series of experiments carried out at the University of Alberta to investigate the pantothenic acid requirements of Yorkshire swine fed small grain rations over a period of two generations.

EXPERIMENTAL

The experimental animals included 4 groups of gilts and representative suckling pigs from the litters of gilts in each

[1] The data reported in this paper are a portion of a thesis presented by Bruce D. Owen as partial fulfillment of the requirements for the degree of Master of Science. Acknowledgments are made to Dr. D. R Clandinin and Dr. A. R. Robblee and Miss R. Renner for invaluable assistance with assay procedure.

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of these groups. The gilts in lot 1 were fed from the time they were weaned at 8 weeks of age on a basal ration of barley supplemented with protein (meat scraps, soybean oil meal, alfalfa meal), minerals (iodized salt and ground limestone) and vitamins A and D. Levels of three, 6 and 12 mg of synthetic calcium pantothenate per pound of feed were added to the rations in lots 2, 3 and 4, respectively.

The control ration was formulated to be as low in pantothenic acid as was feasible using small grains as a basis, and allowing adequate amounts of protein, minerals and vitamins other than pantothenic acid. The ration fed to lot 1 between the middle of gestation and the end of the lactation period contained 14.6% protein on the basis of Kjeldahl nitrogen determination, and 5.5 μg of pantothenic acid per gram of feed on the basis of microbiological assay following sample preparation by a method similar to that of Ives and Strong ('46). This is equivalent to 2.5 mg of pantothenic acid per pound of feed. For growing pigs, the National Research Council ('50) recommends 4.7 mg of pantothenic acid per pound of feed, but at no time were any indications of a pantothenic acid deficiency observed in the control pigs.

Blood and milk samples were obtained from each sow at one week and 6 weeks postpartum. At one week and 6 weeks after birth, blood samples were also taken from two pigs, one of each sex, which were selected as representative of each litter. The method of obtaining blood samples from the anterior vena cava was similar to that used by Carle and Dewhirst ('42), while the milk samples were obtained by the use of oxytocin in a method similar to the one employed by Braude et al. ('46). Samples were frozen rapidly at — 6°F. and stored until such time as the determinations could be made.

The pantothenic acid content of the blood and milk samples was determined microbiologically using *Lactobacillus arabinosus* 17–5 as the test organism. Assays were conducted on a semimicro scale in which the final volume was 2 ml. The composition of the basal medium used and the assay technique followed were similar to those described by Riesen et al.

('47). The medium differed from that used by these workers in that proline was included.

So far as is known, this type of medium, wherein the casein or peptone is replaced by a solution of pure amino acids, has not been used previously for the assay of pantothenic acid. The medium was found very satisfactory as it yielded extremely low blanks and gave a smooth, repeatable standard curve.

Two procedures were used in the preparation of the blood and milk samples for assay: i.e., autolysis in water for 15 minutes in an autoclave at 121°C., and enzymatic hydrolysis. The latter procedure involved digestion of the sample with a fungal enzyme, mylase P. The preparation of the samples for assay was based on the method used by Ray et al. ('47) with several modifications being made to adapt the method to the purpose of this assay. All samples were assayed in duplicate, and the values reported were obtained in at least two replicate assays. Recoveries in almost all of the cases were between 90 and 110%. As no standardized procedure using chicken liver enzyme and intestinal phosphatase for the release of bound pantothenic acid was available while this work was in progress (György, '50), it was not felt advisable to use this method.

RESULTS

The results of pantothenic acid assays carried out on the various blood samples are shown in table 1.

Enzymatic digestion of the samples gave consistently higher values for pantothenic acid than were obtained by water hydrolysis. The increase in values obtained was considerably greater in the case of sows' blood than in the blood of the young pigs. It may be noted also that the pantothenic acid content of the sows' blood increased as the stage of lactation advanced, while levels of the vitamin decreased in the blood of the young pigs as they grew older. There was no indication that the sex of the young pigs had any influence on the pantothenic acid content of their blood. Although blood levels

tended to correlate with the levels of pantothenic acid in the ration, statistically significant differences between lots were not present insofar as the content of pantothenic acid in the blood was concerned.

TABLE 1

Pantothenic acid content of swine blood

LOT NO.	1		2		3		4	
Treatment	CONTROL		CONTROL + SUPPLEMENTAL CALCIUM PANTOTHENATE 3 mg/lb.		6 mg/lb.		12 mg/lb.	
Time after parturition	1 wk.	6 wks.	1 wk.	6 wks.	1 wk.	6 wks.	1 wk.	6 wks.
	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml
				Water hydrolysis				
Sow 1	0.18	0.27	0.18	0.20	0.23	0.32	0.24	0.53
Av. of 2 pigs	2.63	0.71	3.14	0.68	3.27	0.76	3.52	0.84
Sow 2	0.15	0.27	0.27	0.27	0.40	0.47	0.41	0.35
Av. of 2 pigs	1.89	1.27	1.58	1.03	5.75	1.40	2.40	1.65
Sow 3					0.24	0.29	0.39	0.55
Av. of 2 pigs					1.72	1.46	5.19	1.02
Av. of all sows	0.16	0.27	0.23	0.24	0.29	0.36	0.35	0.47
Av. of all pigs	2.26	0.99	2.36	0.85	3.58	1.20	3.70	1.17
				Enzymatic hydrolysis				
Sow 1	0.55	0.69	...	0.63	0.57	0.73	0.67	0.98
Av. of 2 pigs	3.61	1.09	3.82	1.05	4.27	1.17	4.48	1.22
Sow 2	0.38	0.81	0.61	0.76	0.58	0.94	0.61	0.92
Av. of 2 pigs	2.51	2.08	2.06	1.69	6.66	1.92	2.87	2.25
Sow 3					0.47	0.66	0.59	1.08
Av. of 2 pigs					2.34	1.93	6.01	1.43
Av. of all sows	0.46	0.75	0.61	0.70	0.54	0.78	0.62	0.99
Av. of all pigs	3.06	1.58	2.94	1.37	4.42	1.68	4.45	1.63

Results of assays carried out on the milk samples are shown in table 2.

Analysis of variance according to the method of Johnson ('50) was carried out on the data obtained following water hydrolysis of the samples. The results indicated that the pantothenic acid content of the sows' ration had a very definite effect on the amounts of the vitamin present in the milk, as the milk levels increased consistently and quite markedly with the amount of calcium pantothenate added to the basal ration. As was the case in sows' blood, the panto-

thenic acid content of the sows' milk increased as the lactation period progressed. A possible explanation for the lower values for pantothenic acid obtained on the milk samples following enzymatic hydrolysis will be discussed.

TABLE 2

Pantothenic acid content of sows' milk

LOT NO.	TREATMENT	SOW NO.	TIME AFTER PARTURITION			
			1 week		6 weeks	
			Water hydrolysis	Enzyme hydrolysis	Water hydrolysis	Enzyme hydrolysis
			μg/ml	μg/ml	μg/ml	μg/ml
1	Control ration	1	3.99	3.84	3.63	3.97
	(2.5 mg pantothenic	2	7.01	6.34	8.75	6.95
	acid per pound feed)	Av. for lot	5.50	5.09	6.19	5.46
2	Control + 3 mg	1	6.24	5.71	6.66	6.99
	calcium pantothenate	2	5.92	6.03	7.45	6.15
	per pound feed	Av. for lot	6.08	5.87	7.06	6.57
3	Control + 6 mg	1	7.81	7.17	9.30	8.87
	calcium pantothenate	2	9.36	10.19	13.23	11.48
	per pound feed	3	7.18	8.13	9.90	7.70
		Av. for lot	8.12	8.50	10.81 [1]	9.35
4	Control + 12 mg	1	10.14	9.17	17.93	17.15
	calcium pantothenate	2	8.19	9.30	15.78	14.15
	per pound feed	3	10.54	10.78	14.98	12.98
		Av. for lot	9.62 [1]	9.75	16.23 [2]	14.76

[1] Significant difference between this and corresponding value in the control lot.

[2] Highly significant difference between this and corresponding value in the control lot.

DISCUSSION

As is indicated in table 1, the blood levels of pantothenic acid tended to increase in both sows and young pigs with the level of the vitamin in the sows' ration. This trend appeared to be quite regular, although several values were obtained for individual animals which were considerably out of line, and none of the differences was statistically significant. Luecke et al. ('50) observed a similar trend in blood levels of pantothenic acid in swine. The values obtained

following enzymatic digestion of samples were consistently higher than those obtained following water hydrolysis. This would indicate that some of the blood pantothenic acid existed in a bound form, which observation agrees with the work reported by Wright ('43). The increase in values obtained following enzymatic digestion was considerably greater for sows than for young pigs, which possibly indicates that a greater proportion of bound pantothenic acid existed in the blood of the older animals. It may also be noted that the pantothenic acid level in the blood of the sows increased as lactation progressed, while that in the pigs' blood decreased. Although there are no comparable results of other workers with which to compare these findings, a similar occurrence takes place in the case of certain other blood constituents of swine. The work of Grummer et al. ('48) shows a similar trend in blood levels of other members of the B-complex group of vitamins.

The results summarized in table 2 of assays which were conducted on milk samples indicate that the level of pantothenic acid in the sows' ration was reflected in the milk levels of the vitamin. The pantothenic acid in the milk of sows in lot 4 at one week and 6 weeks and in lot 3 at 6 weeks after parturition, was higher by either a significant or highly significant degree than the respective levels in the control lot. Throughout the other lots there was a definite trend toward increased levels of the vitamin in the milk as the level in the ration increased. Differences in milk levels of pantothenic acid tended to be wider than corresponding differences in the blood. This is not surprising, since the blood tends to remain reasonably constant in the level of most nutrients, while secretions and places of storage of various constituents may vary widely in the content of that same nutrient. It was also noted that an increase in milk pantothenic acid levels occurred between one week and 6 weeks after farrowing; an increase which was more marked as the level of the vitamin in the ration was increased. Assays of sows' milk by Davis et al. ('51) also indicated that the level of pantothenic acid

in the milk increased as lactation progressed. Pantothenic acid levels in milk as reported by these workers appear to be lower than those obtained in the present study. This may be explainable on the basis of the different type of basal ration used or by a breed difference in the animals used. However, the differences are probably not of sufficient magnitude to be other than normal variation.

The fact that the values obtained following enzymatic digestion of milk samples were quite consistently lower than those obtained following water hydrolysis is a feature of the milk assay results which is difficult to explain. The differences were greater than those encountered between duplicates of either kind of hydrolysis, so that normal variation is not the explanation. It should be noted here that other workers (Lawrence et al., '46; Davis et al., '51) have reported that little or none of the pantothenic acid in milk is in a bound form, so that the water hydrolysis values obtained in these assays may be considered as maximum. This would indicate that there was perhaps some slight destruction of the vitamin during enzymatic digestion.

SUMMARY

The pantothenic acid content of the blood and milk of sows fed varying levels of pantothenic acid, and of the blood of their suckling pigs, was determined by microbiological assay using *L. arabinosus* as the test organism. In the basal medium, a solution of pure amino acids was used to replace casein or peptone as previously used in pantothenic acid assays. The results illustrated that the pantothenic acid contents of the blood and milk of the sows and of the blood of the suckling pigs were related to the content of the vitamin in the sows' ration. This was particularly true in the case of the milk, where it was found that supplemental levels of 6 or 12 mg of calcium pantothenate per pound of feed fed to the sows resulted in statistically significant increases in the milk levels of the vitamin. Enzymatic digestion using mylase P gave higher pantothenic acid values than water hydrolysis for

sows' and young suckling pigs' blood, but not for the sows' milk.

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FACTORS AFFECTING THE DEVELOPMENT OF ACRODYNIA IN PYRIDOXINE-DEFICIENT RATS

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The first report of a "pellagra-like" condition in rats was made in 1926 (Goldberger and Lillie, '26). Although not recognized as such, this condition was the result of a vitamin B_6 deficiency in rats maintained on a diet which consisted mainly of alcohol-extracted cornmeal. The deficiency syndrome appeared in the animals as evidenced by bilateral symmetrical scaly dermatitis of the ears, extremities, and face. In 1934 and 1935, it was demonstrated that a deficiency of vitamin B_6 produced the symptoms of dermatitis noted by Goldberger and Lillie (György, '34, '35). György suggested that this dietary essential should be named the "rat acrodynia factor" since the lesions of the extremities resembled those observed in human acrodynia, rather than pellagra.

A more detailed description of the rat acrodynia associated with vitamin B_6 deficiency has been given in the literature (Sullivan and Nicholls, '40). Grossly, the initial change is erythema of the dorsa of the paws, most commonly the hind ones, spreading to the plantae, and followed by hyperkeratosis and scaling. The digits next become swollen. Coincident with these changes in the extremities, the same process takes place in the ears, nose, chin, submental region, and occasionally the upper thorax. Although the coat is ill-kempt, very little alopecia is observed until late in the course of

deficiency. Whether involvement of epithelial tissue with subsequent breakdown is a result of secondary infection (Sullivan and Nicholls, '40) or whether it is a primary effect of the deficiency (Wolbach and Bessey, '42) is not yet known.

Several factors have been reported to influence the development of the acrodynia. Within limits, the time of appearance of deficiency symptoms in rats deprived of vitamin B_6 decreased with an increasing protein level in the diet (Cerecedo and Foy, '44). Supplementation of a 15% casein, pyridoxine-deficient diet with methionine or cystine (Cerecedo et al., '48) or with homocystine (Cerecedo and deRenzo, '50) hastened the appearance of these symptoms and increased the severity of the vitamin deficiency syndrome. Pagé and Gingras ('47) have reported that addition of glycine to an 18% casein, pyridoxine-deficient diet provoked a rapid appearance of acrodynia in rats.

The acrodynia of vitamin B_6-deficient rats has been related to the essential fatty acids by several investigators with conflicting conclusions. Quackenbush and associates ('39) reported that the symptoms developed in rats maintained on a pyridoxine-free diet could be alleviated by administration of various natural fats and synthetic esters, the activity of the natural fats for this effect being parallel with their degree of unsaturation. Salmon ('38) has noted that both a heated yeast extract and certain oils are necessary for the maintenance of a normal skin condition in rats, and Birch ('38) has indicated that both fat and water-soluble factors are necessary. In a later study, Salmon ('41) demonstrated that the dermatitis in rats provided with casein, sucrose, salts, carotene, calciferol, alpha-tocopherol, thiamine, riboflavin, and choline could be cured by the administration of pyridoxine, linoleic acid, and pantothenic acid. Quackenbush and associates ('42) later showed that the skin condition which developed in rats fed a highly-purified, fat-free, pyridoxine-free diet could be completely cured by administration of linoleic acid. With subcurative doses of linoleic acid, pyridoxine was

effective, more so in the presence of pantothenic acid than in its absence.

The addition of the vitamin analogue, desoxypyridoxine, decreases the time of appearance, and increases the severity of the acrodynia. With the dietary levels employed, the pattern of the deficiency syndrome appears to be unchanged from that observed by simple pyridoxine deprivation (Stoerk, '50).

The experimental data reported here have been obtained during an investigation of metabolic disturbances in vitamin B_6 deficiency. Since several interesting observations were noted regarding the development of the acrodynia in pyridoxine-deficient rats, and since no correlation was observed between metabolic changes and the appearance of acrodynia, it was felt advisable to describe these findings separately from the data on metabolic changes.

EXPERIMENTAL AND RESULTS

In the experiments to be reported, the analogue, desoxypyridoxine, was employed to facilitate the development of the deficiency syndrome. This was carried out by the addition of 100 μg desoxypyridoxine per rat per day to the purified diets; control animals were further provided with 50 μg pyridoxine hydrochloride per rat per day in their food.

Two types of observations have been made with regard to acrodynia: the length of time required for the first clear-cut signs, and the severity of the lesions at the end of a particular interval. Both types are affected by considerable subjective error. The severity of the acrodynia has been graded as slight, moderate or severe in the following ways: slight acrodynia, erythema of the dorsa of the paws with some evidence of swelling; moderate acrodynia, marked erythema and swelling of the dorsa and plantae of the paws with erythema about the nose; severe acrodynia, marked erythema, swelling, and scaling about the paws, nose, and chin, accompanied by open lesions about the same areas.

A consistent observation which has been noted in the numerous experiments performed in this laboratory has been the greater ease with which male albino rats develop acrodynia as compared with females. Not only is the rate of appearance of acrodynia greater in male rats, but the acrodynia is much more severe after a prolonged experimental period.

Supplementation of an incomplete protein diet with certain amino acids

Albino rats of the Wistar strain, with an average initial body weight of 136 gm were housed in individual, screen-bottomed cages, and supplied with food and water ad libitum. The basal diet contained the following ingredients in per cent by weight: gelatin 16, sucrose 70, agar 2, vitamin powder 4, choline 0.2, inositol 0.2, cod liver oil concentrate 0.015 (calculated to supply 45 I.U. of vitamin A and 11 I.U. vitamin D per rat per day), DL-tryptophan 0.44, L-glutamic acid 3.2, and salts mixture 4 (Steenbock and Nelson, '23). The vitamin powder was prepared by thoroughly mixing 800 gm of gelatin with the following amounts (in milligrams) of vitamins: thiamine chloride 100, riboflavin 100, calcium pantothenate 400, nicotinic acid 400, *p*-aminobenzoic acid 400, biotin 20, and folic acid 20. The inclusion of this vitamin powder brings the total protein content of the diet to 20%. It should be noted that this basal diet is devoid of fat, and is referred to later as "fat-free."

In certain cases, as will be indicated, the following amino acid supplements were added to the diet in per cent by weight at the expense of sucrose: DL-methionine 0.55, and DL-histidine 0.4. These supplements made the methionine and histidine contents of the gelatin diet comparable with a diet differing only in that vitamin-free casein replaced the gelatin again in amount to give a total protein content of 20%. All of the animals were provided with 100 μg desoxypyridoxine per rat per day in their food, and control animals on each diet were further provided with 50 μg pyridoxine per

rat per day in their food. In every case, groups contained 4 male and 4 female rats, and were maintained on experimental diets for a period of 24 days. The results of this experiment are given in table 1.

It would appear that under experimental conditions employing an incomplete protein in the diet, the external sign of pyridoxine deficiency, acrodynia, is not manifested. Even those animals provided with pyridoxine on gelatin-containing

TABLE 1

Effects of an incomplete protein diet on the appearance of acrodynia

DIET	PYRIDOXINE	AVERAGE CHANGE IN BODY WEIGHT	AVERAGE DAILY FOOD INTAKE	ACRODYNIA
		gm.	gm.	
20% casein	+	+ 55	19.9	none
20% casein	—	— 6	12.9	severe
20% gelatin	+	— 23	18.3	none
20% gelatin	—	— 37	14.1	none
+ methionine	+	— 11	17.9	none
+ methionine	—	— 19	11.0	none
+ histidine	+	— 26	12.5	none
+ histidine	—	— 37	10.4	none
+ methionine + histidine	+	— 12	13.6	none
+ methionine + histidine	—	— 29	11.1	none

diets lost weight. This suggests the possibility that acrodynia develops only under dietary conditions which permit an increase in body weight when vitamin B_6 is furnished. It must be noted that when gelatin, methionine, histidine, glutamic acid, and tryptophan are fed conjointly, the amino acid composition of the diet is still inferior to that of the casein diet and the possibility exists that one or more amino acids, either absent or in low concentration, may be responsible for the absence of the acrodynia.

The effect on acrodynia of supplementing a casein diet with glutamic acid

The basal diet employed in this experiment was that described in the gelatin experiment with the one exception that the gelatin was replaced by vitamin-free casein. A second diet was employed differing in that the total casein content was raised from 20% to 75% by weight at the expense of sucrose. The third diet used was identical with the 20% casein diet but L-glutamic acid was added at a level of 12.1% by weight at the expense of sucrose, thus making it comparable

TABLE 2

The effect on the appearance of acrodynia of dietary L-glutamic acid

DIET	PYRIDOXINE	AVERAGE CHANGE IN BODY WEIGHT	AVERAGE DAILY FOOD INTAKE	ACRODYNIA
		gm	*gm*	
20% casein	+	+ 28	17.3	none
20% casein	—	— 7	14.4	slight
75% casein	+	+ 4	12.4	none
75% casein	—	— 24	8.4	moderate
20% casein + glutamic acid	+	+ 22	15.0	none
20% casein + glutamic acid	—	— 24	13.1	severe

in glutamic acid content with the 75% casein diet. Again, 100 μg desoxypyridoxine per rat per day were added to the diet of all animals, and the control animals were further provided with 50 μg pyridoxine hydrochloride per rat per day in their food. Food and water were supplied ad libitum and animals were maintained on these regimes for 20 days. Each group was comprised of 6 male and 6 female rat with an average body weight of 140 gm. The accrued data on acrodynia are given in table 2.

In the literature, pyridoxine has been related to glutamic acid metabolism, particularly in transamination and decar-

boxylation reactions. From the results of this experiment it would appear that supplementation of a casein-containing diet with L-glutamic acid precipitated the development of acrodynia. This effect was more pronounced when glutamic acid was added alone and in the free form, than when it was added in combination with other amino acids to comprise the 75% casein diet. It has already been indicated that the addition of methionine, cystine, homocystine, or glycine, or increasing the protein level of the diet, will provoke a rapid appearance of acrodynia in rats deprived of pyridoxine. It would seem that another amino acid, glutamic acid, has the same marked effect when supplied with a complete protein.

The effect on acrodynia of the inclusion of corn oil in the diet

Since, in the literature, pyridoxine has been related to fat metabolism, it was felt that adding corn oil to a fat-free, pyridoxine-free diet might have a significant effect on the development of acrodynia. As described in the introduction to this paper, the essential fatty acid, linoleic acid, has been reported to alleviate this external deficiency sign; however, little is known of the effect of including several fatty acids in combination (e.g., corn oil) in a pyridoxine-free diet.

In this experiment, three diets were employed: the 20% casein diet already described, the same diet with corn oil added to a level of 5% by weight at the expense of sucrose, and the 20% casein diet with corn oil added to a level of 20% by weight at the expense of sucrose. In all cases the animals were provided with 100 μg desoxypyridoxine per rat per day in their food, and control animals on each diet were further provided with 50 μg pyridoxine hydrochloride per rat per day in their food. Each group was comprised of 5 male and 5 female rats with an average body weight of 211 gm. Each control group was restricted to the same food intake as its comparable deficient group, and all animals were maintained on these dietary regimens for a period of 28 days. The results of this experiment are recorded in table 3.

It was found that corn oil had no effect on the rate of appearance of the acrodynia (21 days), but had a marked effect in preventing the development of a severe degree of the deficiency sign. The effect of corn oil in apparently preventing severe acrodynia is consonant with the concept of an interrelationship between vitamin B_6 and the essential fatty acid, linoleic acid.

TABLE 3

The effect on acrodynia of adding corn oil to a fat-free diet

DIET	PYRIDOXINE	AVERAGE CHANGE IN BODY WEIGHT	AVERAGE DAILY FOOD INTAKE	ACRODYNIA
		gm	*gm*	
fat-free	+	— 9	11.2	none
fat-free	—	— 25	11.2	severe
5% corn oil	+	+ 12	10.9	none
5% corn oil	—	— 35	10.9	slight
20% corn oil	+	+ 32	11.3	none
20% corn oil	—	— 17	11.3	slight

Hormonal effects on the development of acrodynia

The results of several experiments studying the interrelation of pyridoxine and certain hormones will be briefly summarized. Work in this laboratory has suggested that acrodynia does not develop in adrenalectomized rats maintained on the 20% casein, 20% corn oil diet previously described, when provided with desoxypyridoxine but no pyridoxine. In contrast with this observation, similarly treated ovariectomized rats, and intact animals subcutaneously injected with 4 μg of an oestrogenic preparation [1] in olive oil per rat per day, exhibited a typical pyridoxine-deficiency syndrome. It has been further noted that subcutaneous injection of an adrenocorticotrophic hormone preparation,[2] or of an adrenal cortical extract preparation,[3] were without effect on the de-

[1] Ayerst, McKenna, Harrison.

[2] Armour Laboratories 212–103.

[3] Connaught Medical Research Laboratories.

velopment of acrodynia. Daily subcutaneous administration of a growth hormone preparation,[4] on the other hand, appeared to aggravate the pyridoxine deficiency.

SUMMARY

Several factors have been noted during investigations with pyridoxine which affect the development of the external deficiency sign, acrodynia.

1. Rats maintained on a diet containing gelatin as the source of nitrogen do not exhibit acrodynia when deprived of pyridoxine and provided with desoxypyridoxine, even when this diet is supplemented with tryptophan, glutamic acid, methionine, and histidine.

2. Supplementation of a diet containing casein with glutamic acid appears to provoke the development of acrodynia in pyridoxine-deficient rats.

3. The inclusion of corn oil in a fat-free, pyridoxine-free diet had no effect upon the rate of appearance of acrodynia, but did appear to prevent the development of a severe stage of the external deficiency sign.

4. In all of the groups studied the appearance of acrodynia was more frequent and more severe in male rats than in the females.

5. Adrenalectomy appeared to prevent the appearance of acrodynia, whereas ovariectomy, ACTH administration and adrenal cortical extract administration were without effect. Administration of a growth hormone preparation appeared to aggravate a pyridoxine deficiency.

ACKNOWLEDGMENTS

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[4] Armour Laboratories 22KB1.

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INTERMEDIATES FORMED DURING THE DIGESTION OF TRIGLYCERIDES

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ONE FIGURE

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It seems probable that the cleavage of triglycerides in the intestinal tract involves a series of step-wise reactions from triglyceride (TG) to diglyceride (DG) to monoglyceride (MG) and possibly to glycerol (G), with fatty acid (FA) being released at each step. Such DGs could be either the 1,3- or 1,2-isomer, while the MGs could be either the 1- or 2-isomer depending on whether the reaction resulting in the removal of fatty acid(s) from the glyceride molecule are random or selective. On the basis of the composition of the lipids recovered from the thoracic duct of rats fed tagged TGs, Reiser et al. ('52) concluded that the major portion of ingested fat was hydrolyzed to MGs prior to absorption. The high hydroxyl value of the lipids isolated from the lumen of the intestinal tract of rats was interpreted by Frazer and Sammons ('45) as showing the presence of MGs. Although no experimental details are given, a later paper states that MGs were found in the intestinal tract of human subjects (Frazer, '47). Longenecker ('46) has referred to unpublished work carried out by him which demonstrated the formation of MGs and DGs in the intestinal tract during the digestion of fat. The recent development of several new methods for studying partial glycerides, some of which are reported here for the first time, has enabled us to demonstrate with certainty the presence of MGs and DGs in the intestinal tract as the

result of lipolytic action. Moreover, evidence has been obtained which indicates a selective hydrolysis resulting in the formation of 1,2-DGs and 2-MGs.

EXPERIMENTAL

Male rats weighing 100 to 200 gm were fasted 48 hours and then were given 1.5 ml of fat by stomach tube. The animals were sacrificed at the time intervals given in the individual experiments. The intestines, and in some instances the stomach, were removed separately and the contents flushed out with ethyl ether. The ether solution was immediately dried with sodium sulfate and filtered. Separate experiments established that lipase action ceased completely at this point. The solvent was removed under vacuum at 40°C.; the lipid residue was weighed and then taken up in chloroform. This solution of lipids was divided into two equal fractions. One fraction was washed with water and dried with sodium sulfate preliminary to analysis. The other fraction was treated with perchloric acid to convert the 2-MGs to 1-MG. The sample was then washed with water, dried with sodium sulfate, and analyzed.

Under the experimental conditions employed, 200 to 300 mg of lipid were usually recovered from the lumen of the intestine of a rat which had been fed TG two to 4 hours earlier. The small quantity of material available for analysis necessitated the modification of several standard macro methods. The procedure used for determining MGs was essentially that described by Pohle and Mehlenbacher ('50) except that the solution concentrations were reduced. With this method pure TGs, 1,2-DGs, 1,3-DGs, and 2-MGs yielded blank values of up to 1.5% apparent 1-MG. Thus, MG values below 1.5% are considered not to be significant under the experimental conditions employed here. Similar observations with respect to such blank values have been reported by Kummerow and Daubert ('50). With increasing amounts of 1-MG in the lipid being studied, the size of the sample used for analysis can be reduced and hence the non-specific reaction of TGs

and DGs with periodic acid becomes of less significance. On known mixtures of glycerides, it was found that when the concentration of 1-MG in the lipid is in excess of 6%, the experimentally obtained values are in close agreement with the true values. Although 2-MG does not react with periodic acid, it was possible, by determining the amount of 1-MG initially present, isomerizing the 2-MG to 1-MG, and then again analyzing for 1-MG, to calculate by difference the amount of 2-MG initially present.

Free FAs were determined by titration. Iodine values were determined by a micro adaptation of the Wijs method. Hydroxyl values were obtained by a modification of the Zerewitinoff active hydrogen method using lithium aluminum hydride as the reagent (Hochstein, '49). The quantity of evolved hydrogen was corrected for that contributed by the free FA; the residual value was then used in calculating the hydroxyl value of the sample. The quantity of DG present was calculated from the hydroxyl value remaining after subtracting from the total hydroxyl value the number of hydroxyl units equivalent to the total MG present.

Craig separation runs were made in a 54-tube apparatus using heptane-80% ethanol as the solvent pair (Zilch and Dutton, '51). Under these conditions the MG fraction peaks at tube 9, free FAs at tube 40, and the DGs and TGs at tube 52. The partition coefficients of 1-MGs and 2-MGs were found to be essentially identical.

Studies with synthetic 2-MG demonstrated that conversion to the 1-isomer could be brought about by a number of different methods. Of these, the one chosen as being the most efficient, and yet causing the minimum of hydrolysis and interesterification reactions, was treatment with perchloric acid. Essentially, the method consists of dissolving the lipids in chloroform so as to obtain approximately a 1% solution of MGs. To this is added sufficient 55% perchloric acid to obtain a ratio of one part perchloric acid for every 4 parts of MG. The solution is mixed and allowed to stand at room temperature for 30 minutes. Under these conditions there is

a 70 to 85% conversion of the 2-isomer to the 1-isomer. Since this treatment does not bring about complete isomerization, the values for total MG, as determined by the periodic acid method, are minimal. The variation in the extent of isomerization makes it impracticable to correct the observed values. On the other hand, the DG values will be maximal, since the hydroxyl value from which the quantity of DG is calculated must be corrected for the quantity of MG present.

Although not used as the method of isomerization, heat also causes a conversion of 2-MG to 1-MG. Thus holding a sample of 2-MG at a temperature of 100°C. for three hours will cause as much as 80% isomerization. At lower temperatures, the rate of conversion is slower. The variations in the extent of isomerization, and the accompanying hydrolytic and esterification reactions, when heat is used, make it an unsuitable method.

RESULTS

In the first series, 12 rats were given 1.5 ml of partially hydrogenated vegetable oil. Groups of three rats each were sacrificed at zero, two, three, and 4 hours. The intestinal and stomach samples of each group of three animals were pooled separately. The MG and free FA content of the isolated lipids are given in table 1.

It will be noted that little or no MG or FA was present in the stomachs of these animals. Similarly, appreciable quantities of digestion products or intermediates could not be demonstrated in the intestines of the zero hour animals. In the intestines of the animals sacrificed at the end of the other time periods considerable amounts of MGs and free FA were present. An appreciable portion of the MGs was of the 2-configuration.

In order to have larger samples for analysis, each of 20 rats was administered 1.5 ml of partially hydrogenated vegetable oil. All of the animals were sacrificed at the end of three hours. The lipids recovered from the lumen of the intestines of these rats were pooled into a single sample.

Analyses of the isomerized and unisomerized samples are given in table 2.

The Craig countercurrent distribution curves are given in figure 1 and the average composition of the lipids as calculated from the curves is presented in table 2. The dotted

TABLE 1

The monoglyceride (MG) and free fatty acid (FA) content of the lipids recovered from the lumen of the stomachs and intestines of rats at various intervals following the feeding of triglyceride [1]

HOURS	FREE FA	1-MG	TOTAL MG	2-MG [2]
	%	%	%	%
		Stomach		
0	2.9	0.7	0.6	0
2	3.0	1.0	0.9	0
3	4.8	1.4	1.5	0
4	1.4	1.8	2.3	0.5
		Intestine		
0	4.2	..	..	1.8
2	21.5	3.4	7.5	4.1
3	24.5	3.2	8.2	5.0
4	35.7	9.6	16.4	6.8

[1] A 75/25 mixture of soybean oil and cottonseed oil hydrogenated to an iodine value of 80.

[2] Determined by difference.

TABLE 2

The percentage composition of the lipids recovered from the lumen of the intestines of rats fed triglyceride [1]

	CHEMICAL ANALYSES	CRAIG SEPARATOR
1-Monoglyceride	3.2	
Total monoglyceride	7.5	10.6
2-Monoglyceride	4.3 [2]	..
Free fatty acid	15.5	13.6
Hydroxyl value [2]	57	..
Diglyceride	36	} 75.5
Triglyceride	41 [3]	

[1] A 75/25 mixture of soybean oil and cottonseed oil hydrogenated to an iodine value of 80.

[2] Theoretical hydroxyl values for partial glycerides of this fatty acid composition are 317 for monoglyceride and 91 for diglyceride.

[3] Determined by difference.

lines on the curves are the areas used in calculating the quantities of FAs, DGs, and TG

The material contained in tubes zero to 20 from the solvent separation of the unisomerized sample was combined into a single sample, hydrogenated to a zero iodine value, and an x-ray diffraction pattern obtained. The pattern was typical

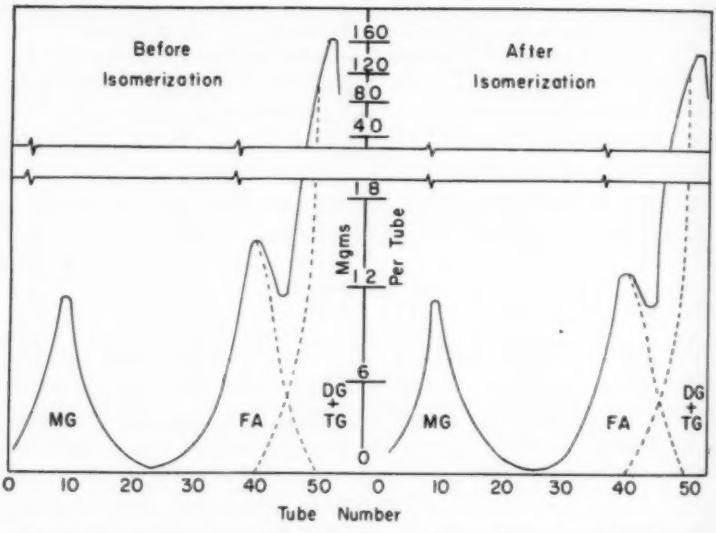

Fig. 1 Countercurrent distribution curves of the lipids isolated from the intestines of rats fed triglyceride.

of that for 1-MG. The presence of 2-MG in the sample will not influence the pattern since during hydrogenation 2-MG is converted to 1-MG.

The presence of MG in the original samples and in the MG fraction isolated by countercurrent distribution was confirmed further by infrared spectrophotometry. The regions of the spectrum where MGs show characteristic absorption are 9.5 and 3 μ.

In the final series, each of 20 rats was given 1.5 ml of 2-oleyldipalmitin (POP), iodine value = 30. All animals were

sacrificed at the end of three hours. The lipids recovered from the lumen of the intestines of these rats were pooled into a single sample. Analyses of the isomerized and unisomerized samples are given in table 3.

The samples were then processed by countercurrent distribution. The distribution curves, although not shown here, were similar to those in figure 1. The MGs contained in tubes zero to 20 were combined into a single sample. The iodine value of this material was 64. The lipids contained in

TABLE 3

The percentage composition of the lipids recovered from the lumen of the intestines of rats fed 2-oleyl-dipalmitin

	CHEMICAL ANALYSES
1-Monoglyceride	6.5
Total monoglyceride	13.3
2-Monoglyceride	6.8 [2]
Free fatty acid	19.7
Hydroxyl value [1]	65
Diglyceride	22
Triglyceride	45 [2]
Iodine value of monoglycerides	64
Iodine value of fatty acids	5

[1] The theoretical hydroxyl value of 326 for monoolein and 94 for 1-palmito-2-olein used in these calculations.

[2] Determined by difference.

tubes 35 to 43 (these would be predominantly free FA with some DGs and TGs) were combined into a single sample. The free FAs were removed by washing with a dilute solution of potassium carbonate. The potassium carbonate wash was acidified and the FAs were recovered by extraction with petroleum ether. These FAs had an iodine value of 5.

DISCUSSION

The analytical techniques used in these experiments had certain limitations which could not be completely overcome. As mentioned, the periodic acid reagent indicates a small amount of apparent MG in fats known to be free of MG. For

this reason the values reported in table 1 for the MG content of the lipids of the stomachs are probably spurious values. The only exceptions to this may be the values at the end of 4 hours. In the case of the figures for the intestinal samples in table 1, except for the zero hour samples, the MG values obtained are well in excess of any limitations the method may have. This is particularly true of values in excess of 6% MG, where the values will be accurate as well as real.

The method of converting 2-MG to 1-MG was not quantitative. Under the conditions employed, the treatment of a pure sample of 2-MG with perchloric acid will cause an 85% conversion to the 1-isomer. However, when TG, DG, and FA are also present this conversion may be as low as 70%. Therefore, in the samples studied here it is possible that only a 70% conversion was obtained. That the isomerization is incomplete is confirmed by the higher values for MG obtained by countercurrent fractionation. Moreover, the MG fractions obtained from the Craig runs on the isomerized samples when analyzed for MG by the periodic method show only 70% 1-MG. The uniformity of the MG fraction of the Craig curves and the fact that 1- and 2-MG have the same partition coefficients in the solvent pair used, indicate the remaining portion of the sample to be 2-MG. It is unlikely that there were other materials in the samples that would concentrate in the zero to 20-tube region.

These limitations in the methods made it desirable to have an additional confirmation of the presence of 2-MG. For this reason the experiment using POP was carried out. If the hydrolysis to MG was a random reaction, then the MG fraction should consist of two parts monopalmitin and one part monoolein. Such a mixture would have an iodine value of 24. If only 1-MG were formed then the iodine value of the MG fraction would be zero, while if only 2-MG were formed then the iodine value would be that of monoolein, 71. The iodine value of 64 that was obtained on the isolated MG was only 10% less than that for pure monoolein. Similarly if the hydrolysis of TGs was a random reaction then the free FAs

should consist of two parts palmitic and one part oleic acid. Such a mixture would have an iodine value of 32. The iodine value of 5 found for the free FAs indicates the predominant free FA to be palmitic acid. Thus from the composition of the MGs formed and the FAs released following the feeding of POP, it appears that the MG resulting from the *in vivo* hydrolysis of TG is predominantly the 2-isomer.

The results obtained in the experiment where POP was fed indicate that some 90% of the MGs formed were of the 2-configuration. However, in none of the experiments did the 2-isomer, as measured by chemical means, ever constitute more than 70% of the total MG present and was usually considerably less. Thus it seems that even in the samples that were not treated with perchloric acid, some isomerization had taken place. Although a small part of this isomerization may have taken place during isolation of the samples, isomerization in the intestine also seems to take place. The increasing amount of the 1-isomer found in the intestinal tract with increasing time (see table 1) indicates such a conversion. An interesting contrast is that in *in vitro* digestion experiments with pancreatic lipase, using the periodic acid method of analysis, we have been able to identify only 1-MG.

The net effect of the limitations described results in the obtaining of minimum values for the MG content. The presence of these minimal quantities of MG has been established by the periodic acid method and countercurrent separation and their presence qualitatively confirmed by x-ray diffraction pattern and absorption in the infrared. Future refinements of technique should have the effect of showing more total MG and 2-MG, and less DG.

Since the MGs initially formed seem to be predominantly of the 2-configuration, as much as 90% being indicated by the experiment when POP was fed, it is reasonable to assume that the DGs formed are the 1,2-isomer. Unfortunately, there is not a suitable method for distinguishing 1,2-DGs from 1,3-DGs.

SUMMARY

1. Lipids recovered from the lumen of the intestines of rats following the feeding of triglyceride were analyzed for monoglyceride by periodic acid before and after isomerization with perchloric acid (this causes a conversion of 2-monoglyceride to 1-monoglyceride), for free fatty acid, and for hydroxyl value. The lipids were also fractionated by the Craig separator and the presence of monoglycerides confirmed by x-ray diffraction patterns and infrared absorption.

2. Appreciable quantities of monoglyceride and diglyceride are formed and accumulate in the lumen of the intestinal tract during the digestion of fat. These glycerides constituted as much as 16 and 36%, respectively, of the total lipids. A portion of the monoglyceride found was of the 2-configuration, and the data indicate that almost all of the monoglycerides formed were initially the 2-isomer. It is likely that the diglycerides formed are of the 1,2-configuration.

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STUDIES OF LIPOGENESIS IN CERTAIN B-VITAMIN DEFICIENCIES [1]

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There is evidence to suggest that many vitamins of the B-complex are concerned with fat metabolism (McHenry and Cornett, '44). The role of choline as a lipotropic factor is well established (Best and Lucas, '43; McHenry and Patterson, '44). It has also been shown that high fat diets spare dietary thiamine (Evans and Lepkovsky, '35; Banerji, '40), and that large doses of dietary biotin may produce cholesterol-rich fatty livers (Gavin and McHenry, '41). Coenzyme A, which contains pantothenic acid (Lipman et al., '47), has been found to be involved in the synthesis of ergosterol and fatty acids from acetic acid in yeast (Klein, '51). The accumulation of cholesterol in livers of rats fed a cholesterol-rich diet was found to be depressed in pantothenic acid deficiency (Morgan and Guehring, '51). In addition, Cheldelin et al. ('51) have reported that livers from pantothenic acid-deficient rats fail to oxidize butyrate and caproate.

Other workers have demonstrated conclusively that acetate is a precursor of fatty acids and cholesterol (Rittenberg and

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Bloch, '45; Bloch, Borek and Rittenberg, '46). This study deals with the effect of thiamine, biotin, pantothenic acid and choline deficiencies upon the incorporation of acetate carbon into fatty acids and cholesterol of liver, blood, heart, and adrenals. The endeavor suggests that there is no defect in lipogenesis in any of these deficiencies.

MATERIALS AND METHODS

Groups of young male rats were given one of the diets shown in table 1. Crystalline vitamins were added in the following quantities per kilogram of diet: riboflavin, 8 mg; pyridoxine, 4 mg; calcium pantothenate, 20 mg; niacin, 20 mg; and thiamine, 4 mg. Diet A was fed ad libitum to the control rats. For production of thiamine and pantothenic acid deficiency the respective vitamins were omitted from diet A. Biotin deficiency was produced by diet B, and choline deficiency by diet C. The control groups which were pair-fed with the thiamine, pantothenic acid and choline-deficient rats were given the same diet as the deficient rats with addition of the missing vitamins; the rats pair-fed with the biotin-deficient rats were offered diet A.

TABLE 1

Composition of diets

	A	B	C
	%	%	%
Casein (vitamin-free)	18	..	12
Egg white	..	20	..
Dextrose	72.5	70.5	78.8
Corn oil	3	3	3
Cod liver oil	2	2	2
Choline chloride	0.3	0.3	..
Cystine	0.2	0.2	0.2
Salts[1]	4	4	4

[1] Hegsted, Mills, Elvehjem and Hart ('41).

A summary of the experimental course of the animals used in this study is shown in table 2. After the characteristic signs

of deficiency had developed, each rat was given daily over three days 0.1 millimoles of radioactive carboxyl-labeled acetate mixed in the food per day per 100 gm of body weight. At the end of the three days of feeding, the animals were killed and their liver, heart, adrenals and blood serum examined. The organs were digested at 70°C. during two hours in 5 ml of 30% KOH, and 5 ml alcohol. After saponification,

TABLE 2

Course of deficiency

DIET	NO. OF RATS	WEIGHT AT BEGINNING OF EXPER.	TIME ON DIET	WEIGHT AT BEGINNING OF TEST	DAILY FOOD INTAKE DURING TEST
		gm	*weeks*	*gm*	*gm*
Normal	20	40	5–6	135	13.7
Pantothenic acid deficiency	20	55	8–12	99	6.7
Pair-fed	16	55	8–12	124	6.9
Thiamine deficiency	11	53	3–4	90	4.8
Pair-fed	12	53	3–4	106	5.0
Biotin deficiency	11	45	6–7	121	10.0
Pair-fed	13	46	6–7	143	10.0
Choline deficiency	11	40	2–3	98	11.0
Pair-fed	12	39	2–3	99	11.1

5 ml of 6N HCl were added. The fatty acids and cholesterol were extracted with ether, the ether evaporated, and the residue transferred in small centrifuge tubes. After the addition of 3 ml of a 1:1 alcohol-acetone mixture, cholesterol was precipitated with digitonide. On the following day the precipitate was washed with acetone-ether (2:1) and then ether, and the fatty acids of the liver determined gravimetrically in the supernatant solution. The precipitate was dried and an aliquot examined for cholesterol according to the methods of

Sperry ('38) and Sperry and Webb ('50). A weighed amount of the dried fatty acids and the part of the cholesterol-digitonide which was not used for cholesterol determination was placed on planchets and counted for radioactivity with an end-window Geiger-Muller counter.

The administration of 1.0 millimoles of acetate per 100 gm of rat per day to one-half the pantothenic acid-deficient and control groups did not significantly affect the radioactivity of the fractions studied, and these data are included in the average for the groups of this study.

RESULTS

Thiamine deficiency

The data obtained with thiamine-deficient rats are presented in table 3. Marked differences were not found in the fatty acids and cholesterol concentrations of the liver, heart, and blood serum between thiamine-deficient rats and their ad libitum and pair-fed controls. A significant decrease in total incorporation of radioactive carbon in liver fatty acids and cholesterol and in heart cholesterol was noted in the deficient animals as well as in the pair-fed controls, whereas the specific activity (counts per minute per milligram fatty acids and cholesterol) remained markedly constant in all groups. The differences in total activity are, therefore, due to the diminished total amount of these substances. It is noteworthy that the pair-fed controls behaved like the deficient animals, which suggests that it is lack of calories rather than thiamine deficiency which caused the depression. The total and relative cholesterol content of the adrenals was considerably diminished in the animals with restricted food intake. The pair-fed group also showed a diminished incorporation of radioactive carbon in adrenal cholesterol, even on a weight basis, which differed significantly from the normal controls.

Pantothenic acid deficiency

Table 4 shows that pantothenic acid deficiency does not affect fatty acid and cholesterol concentrations of the liver,

TABLE 3

Thiamine deficiency

(Weight, fatty acid and cholesterol content of liver, heart, adrenals, and blood serum)

CONDITION	NO. OF RATS	LIVER				HEART		ADRENALS			BLOOD SERUM
		Fatty acids, gm		Cholesterol, mg		Cholesterol, mg		Weight	Cholesterol, mg		Cholesterol
		total	*per 100 gm*	*total*	*per 100 gm*	*total*	*per 100 gm*	*mg*	*total*	*per 100 gm*	*mg %*
Normal	10	0.202	2.56	18.12	242	0.64	117	32	0.50	1579	49
Deficient	11	0.071	2.04	8.02	211	0.43	114	31	0.44	1410	52
Pair-fed	12	0.092	2.05	9.45	210	0.50	115	30	0.35	1158	44

Counts per minute/100.000 c/m given, means and standard deviations

CONDITION	LIVER				HEART		ADRENALS		SERUM
	Fatty acids		Cholesterol		Cholesterol		Cholesterol		Cholesterol
	per mg	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*
Normal	12 ± 5.5	2068 ± 965	22 ± 5.0	341 ± 74	11 ± 2.3	7 ± 1.6	15 ± 6.9	5 ± 2.4	15 ± 4.9
Deficient	15 ± 8.8	1095 ± 517	20 ± 5.0	163 ± 46	9 ± 4.4	4 ± 2.3	11 ± 7.2	4 ± 2.9	16 ± 3.9
Pair-fed	14 ± 4.3	1242 ± 488	20 ± 4.2	186 ± 65	9 ± 3.1	5 ± 1.9	10 ± 3.9	3 ± 1.7	17 ± 8.5

heart, and blood serum, although the total incorporation of C^{14} in these lipids is reduced. The differences in the specific activity, however, were small and insignificant. The adrenals of the deficient animals contained considerably less, and those of the pair-fed controls much more cholesterol than those of the normal animals. As in the case of thiamine deficiency, both deficient and pair-fed rats had a low specific activity in adrenal cholesterol, and the total counts were normal in the pair-fed, and low in the deficient groups.

Biotin deficiency

In this type of deficiency great differences in the fatty acid and cholesterol concentration of the liver, heart, and blood serum were not observed between the deficient animals and their ad libitum and pair-fed controls (table 5). In contrast to the thiamine-deficient and the pantothenic acid-deficient rats incorporation of C^{14} into liver fatty acids by deficient and pair-fed rats was normal, and only the cholesterol synthesis was reduced. Again there is no specific effect of deficiency, although the better food intake in the deficient group slightly modified the results. Adrenal cholesterol concentration was not affected by biotin deficiency, although the incorporation of radiocarbon in adrenal cholesterol was found to be diminished in both the deficient and pair-fed rats.

Choline deficiency

As was expected a considerable increase in the liver fatty acids was found in the choline-deficient animals (table 6). The choline content of the diet of the pair-fed group (which differed from that of the normal control group by its low protein content) did not entirely prevent the rise in fatty-acid content above normal levels. Both deficient and pair-fed animals showed a diminished cholesterol content and concentration in the heart, adrenals, and blood serum, liver cholesterol being subnormal in the pair-fed group.

TABLE 4

Pantothenic acid deficiency

(Weight, fatty acid and cholesterol content of liver, heart, adrenals, and blood serum)

CONDITION	NO. OF RATS	LIVER				HEART		ADRENALS			BLOOD SERUM
		Fatty acids, gm		Cholesterol, mg		Cholesterol, mg		Weight	Cholesterol, mg		Cholesterol
		total	*per 100 gm*	*total*	*per 100 gm*	*total*	*per 100 gm*	*mg*	*total*	*per 100 gm*	*mg %*
Normal	24	0.194	2.46	18.25	231	0.68	123	33	0.47	1478	56
Deficient	20	0.101	2.25	9.94	221	0.57	125	34	0.36	1037	53
Pair-fed	16	0.107	2.32	9.52	207	0.59	113	32	0.66	1990	53

Counts per minute/100.000 e/m given, means and standard deviations

CONDITION	LIVER				HEART		ADRENALS		SERUM
	Fatty acids		Cholesterol		Cholesterol		Cholesterol		Cholesterol
	per mg	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*
Normal	13 ± 4.6	2238 ± 875	21 ± 6.1	337 ± 84	13 ± 4.7	8 ± 3.2	16 ± 7.1	7 ± 3.5	19 ± 7.4
Deficient	11 ± 0.9	1034 ± 376	20 ± 5.0	209 ± 58	12 ± 5.1	6 ± 3.0	12 ± 5.8	4 ± 3.1	22 ± 9.2
Pair-fed	11 ± 0.9	1274 ± 351	18 ± 5.6	177 ± 62	11 ± 7.5	7 ± 3.7	9 ± 4.7	7 ± 3.5	19 ± 14.4

TABLE 5

Biotin deficiency

(Weight, fatty acid and cholesterol content of liver, heart, adrenals, and blood serum)

CONDITION	NO. OF RATS	LIVER				HEART		ADRENALS			BLOOD SERUM
		Fatty acids, gm		Cholesterol, mg		Cholesterol, mg		Weight	Cholesterol, mg		Cholesterol
		total	*per 100 gm*	*total*	*per 100 gm*	*total*	*per 100 gm*	*mg*	*total*	*per 100 gm*	*mg %*
Normal	10	0.202	2.56	18.12	242	0.64	117	32	0.50	1579	49
Deficient	11	0.142	2.58	13.04	237	0.66	115	43	0.65	1547	42
Pair-fed	13	0.146	2.24	13.52	208	0.66	105	33	0.59	1730	45

Counts per minute/100.000 c/m given, means and standard deviations

CONDITION	LIVER				HEART		ADRENALS		SERUM
	Fatty acids		Cholesterol		Cholesterol		Cholesterol		Cholesterol
	per mg	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*
Normal	12 ± 5.5	2068 ± 965	22 ± 5.0	341 ± 74	11 ± 2.3	7 ± 1.6	15 ± 6.9	5 ± 2.4	15 ± 4.9
Deficient	12 ± 4.5	1698 ± 701	19 ± 6.4	251 ± 93	9 ± 3.9	6 ± 2.7	11 ± 6.0	8 ± 5.2	17 ± 5.6
Pair-fed	10 ± 4.9	1405 ± 599	17 ± 9.9	224 ± 47	8 ± 3.3	5 ± 2.1	9 ± 3.4	6 ± 4.2	12 ± 6.7

TABLE 6

Choline deficiency

(Weight, fatty acid and cholesterol content of liver, heart, adrenals, and blood serum)

CONDITION	NO. OF RATS	LIVER				HEART		ADRENALS			BLOOD SERUM
		Fatty acids, gm		Cholesterol, mg		Cholesterol, mg		Weight	Cholesterol, mg		Cholesterol
		total	*per 100 gm*	*total*	*per 100 gm*	*total*	*per 100 gm*	*mg*	*total*	*per 100 gm*	*mg %*
Normal	10	0.202	2.56	18.12	242	0.64	117	32	0.50	1579	49
Deficient	11	0.715	11.92	14.95	245	0.46	103	22	0.26	1149	30
Pair-fed	12	0.190	3.22	10.20	183	0.44	87	23	0.28	1313	31

Counts per minute/100,000 c/m given, means and standard deviations

CONDITION	LIVER				HEART		ADRENALS		SERUM
	Fatty acids		Cholesterol		Cholesterol		Cholesterol		Cholesterol
	per mg	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*	*total*	*per mg*
Normal	12 ± 5.5	2068 ± 965	22 ± 5.0	341 ± 74	11 ± 3.3	7 ± 1.6	15 ± 6.9	5 ± 2.4	15 ± 4.9
Deficient	8 ± 4.6	6063 ± 3040	22 ± 4.8	325 ± 76	11 ± 3.3	5 ± 4.4	11 ± 4.4	3 ± 1.8	20 ± 8.0
Pair-fed	9 ± 2.2	1741 ± 766	22 ± 3.6	232 ± 82	9 ± 2.4	4 ± 0.7	9 ± 3.0	3 ± 1.6	18 ± 12.1

It is noteworthy that no significant difference was found in the specific activity of liver fatty acids and the liver, heart, and serum cholesterol in deficient and normal rats. The total amount of radioactivity was significantly higher in the liver fatty acids of the deficient rats and significantly lower in liver cholesterol of the pair-fed group as a consequence of the higher fatty acid content in choline deficiency and the lower cholesterol content in the pair-fed rats. The total activity of heart and adrenal cholesterol was diminished in the deficient and pair-fed groups. As in the case of other deficiencies, the total C^{14} content of the adrenal cholesterol was low in the deficient and pair-fed groups.

DISCUSSION

The incorporation of radiocarbon from acetate into fatty acids and cholesterol of the liver, heart, and serum of rats was not *specifically* altered by thiamine, pantothenic acid, biotin, or choline deficiencies. There were, however, significant variations in the *total* incorporation of radiocarbon in these organs in some of these deficiencies. There was an increase in total radioactivity in the fatty acids of the liver in choline deficiency, a decrease in that of the liver fatty acids and cholesterol in thiamine and pantothenic acid deficiencies, and a decrease in that of liver cholesterol in biotin deficiency. In all cases except choline deficiency, the pair-fed controls presented similar findings, suggesting that this effect was due to the diminished food intake. It is of interest to note that the greatest decrease in incorporation occurs in thiamine deficiency which is accompanied by the most severe voluntary restriction of food intake (table 2). In this deficiency, the incorporation of C^{14}, even in heart cholesterol, is significantly depressed. In pantothenic acid deficiency the food intake is less drastically reduced, and the decrease in radioactivity of total heart cholesterol is on the borderline of statistical significance. On the other hand, in biotin deficiency, with an almost normal food intake, no significant decrease in total radioactivity was found in liver fatty acids

with reduction only of total liver cholesterol activity. This finding is not in agreement with the claims of Curran ('50) based on 4 rats only, that the synthesis of cholesterol is normal and that of fatty acids slightly increased in this deficiency.

The findings with choline deficiency are of interest in view of the fact that the rate of accumulation of radioactivity exceeded the rate of accumulation of total fat. Two to three weeks are required to produce the observed increase in liver fat while only three days of feeding radioacetate labeled the increased depot of fat as a normal specific activity. This suggests that the inability to metabolize newly synthesized fat becomes increasingly difficult with progressive choline deficiency.

The lack of a specific effect of pantothenic acid deficiency is puzzling, since a specific decrease in incorporation of radiocarbon from acetate might be expected on the basis of the proven role of coenzyme A in fatty acid synthesis in bacteria (Stadtman, '50). The findings of Olson and Kaplan ('48) that even prolonged pantothenic acid deficiency does not completely deplete the rat organism of its coenzyme A content lends an explanation to our results. One might postulate that a certain minimum level of coenzyme A is essential for certain metabolic processes such as lipogenesis, and if this minimum (about one-third normal) is maintained, no metabolic defect is demonstrable. One might postulate further that lowering of coenzyme A below this level as a result of prolonged deficiency results in death rather than further derangement of lipogenesis or other coenzyme A-dependent reactions. These postulates are not necessarily inconsistent with the findings that deficient rats are unable, even at an earlier stage of pantothenic acid deficiency, to utilize a coenzyme A-dependent reaction if a load is imposed, i.e., the acetylation of PABA (Riggs and Hegsted, '48).

Our findings with adrenal cholesterol deserve a further comment. It is believed that adrenal cholesterol may serve as a precursor of cortical hormones (Long, '47) and that its

amount reflects the reaction of the organism to "stressor agents." During the period of initial alarm, the concentration of cholesterol in the adrenals decreases; in the stage of exhaustion it decreases again (Selye, '50). The decreased adrenal cholesterol content of the rats pair-fed with thiamine-deficient rats (severe food restriction), the markedly increased cholesterol concentration in the rats pair-fed with pantothenic acid-deficient rats (moderate food restriction) and the slightly increased cholesterol content in rats pair-fed with biotin-deficient rats (slight food restriction) might find an explanation on the above mentioned basis. In this connection it is noteworthy that while the deficient animals had adrenal cholesterol of normal specific activity, the pair-fed rats of all groups showed a lower specific activity than the normal controls. In pantothenic acid-deficient rats a much lower adrenal cholesterol content was found, thus confirming earlier cytological evidence of a depletion of lipid material from the zona fasciculata in this nutritional disorder (Ralli and Graef, '44; Deane and McKibbin, '46; Melampy, Cheng and Northrop, '51).

SUMMARY

Thiamine, pantothenic acid, biotin, and choline deficiency were produced in young male rats. Experimental animals with ad libitum and pair-fed controls were given radioactive carboxyl-labeled acetate in their food over a period of three days. At the end of this time the animals were killed and their livers examined for fatty acids and cholesterol, and their heart, adrenals, and blood serum analyzed for cholesterol. C^{14} incorporation in fatty acids and in cholesterol was determined.

In none of the nutritional disorders studied here was any effect of the deficiency upon the specific activity of liver fatty acids and liver, heart, and serum cholesterol noted. Total incorporation of radioactive carbon in the liver fatty acids and cholesterol varied as a result of changes in the absolute amount of these lipids in liver.

The specific activity of adrenal cholesterol was found to be decreased in all types of deficiencies and also in the pair-fed controls.

Pair-fed controls behaved like their respective deficient groups except with respect to total liver fatty acids and activity in the choline-deficient series.

These results support the view that lipogenesis per se is not specifically altered in these deficiencies.

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[4] Given erroneously in original published title as "pathogenic."

BASAL METABOLISM OF THE ESKIMO

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It has been claimed by several workers that there are distinct racial differences in basal metabolism (Wilson, '45). No satisfactory explanation has been offered for these differences. In some races, such as Eskimos and American Indians, basal metabolic rates (BMR), significantly higher than the figures considered normal for Whites, have been consistently observed. In the case of Eskimos, figures between 13% and 33% over the DuBois standard have been reported by the greater majority of previous investigators (Heinbecker, '28; Rabinowitch and Smith, '36; Crile and Quiring, '39; Höygaard, '41; Bollerud et al., '50).

In connection with studies on human adaptation to cold which were in progress at the Arctic Aeromedical Laboratory, it was considered desirable to examine, under carefully controlled conditions, whether there is any difference in the basal heat production in Eskimos as compared with Whites, and if so, how such a difference may be explained. On this basis, a series of comprehensive studies of basal metabolism has been carried out from 1950 to 1952 on a large number of Eskimos from various native settlements in Alaska. The purpose of this paper is to report briefly the main results of these studies.

MATERIAL AND METHODS

A total of 340 basal metabolism tests were made on 73 healthy Eskimos (52 males and 21 females) from 4 different locations in Alaska, representing different climatic conditions,

living habits and diets. The first group at Barter Island, which is located on the north coast of Alaska, live on a diet consisting of approximately 50% sea mammals and fish, and 50% land mammals. The second group at Anaktuvuk Pass, located in the middle of the Brooks Range 3,000 feet above sea level, live almost exclusively on land mammals, and especially caribou meat. The third group at Kotzebue, situated on the west coast of Alaska, live to a considerable extent on White man's food, and their living habits are more affected by civilization than any of the other groups. The 4th group at Gambell, St. Lawrence Island, live almost exclusively on sea mammals, and especially walrus meat. Of these groups, the Anaktuvuk Pass group had the most limited access to the White man's food.

These 4 groups were examined both in the winter and in the summer. In addition to the field studies, representative subjects of each group have been studied under carefully controlled conditions in our laboratory at Ladd Field while living on the White man's diet. These studies have included environmental surveys, measurements of body surface area, nutritional surveys with particular reference to protein intake, and urinary nitrogen elimination. In some cases, the respiratory quotient was determined.

The subjects, between 20–40 years old, were carefully selected on the basis of complete medical examination, including medical histories, physical examination with x-rays of the chest and long bones, as well as urine and blood examinations. The body weight had been checked several weeks prior to the test to insure that no significant loss of weight had occurred which might influence the results. A total of 28 normal Whites were used as controls.

At each of the 4 Eskimo settlements a complete and well-equipped field laboratory was established, with adequate sleeping quarters for the Eskimo subjects. The standard technique was applied, and all basal metabolism tests were made with the same Benedict-Roth metabolism apparatus. It was tested regularly for leaks, and great care was taken to make the

subjects accustomed to the apparatus prior to the test. Each test consisted of two 9-minute periods. The technique was checked repeatedly both in the field and in the laboratory. We were fortunate in having Dr. E. F. DuBois as a consultant on these studies, and in one case (E. F. D. B.) we were able to compare our results with those obtained from the same subject in the calorimeter at the Russell Sage Institute of Pathology. Our figures came within 1% of the average for the respiration calorimeter. As a further check on our methods, the author's basal metabolism was examined repeatedly, both in our laboratory at Ladd Air Force Base, and in the field. The results in the field were all within 1% of his average.

The subjects were usually examined in groups of 5 on three successive days. Following the last meal at 5:00 or 6:00 o'clock in the evening, the subjects were admitted to the sleeping quarters at the field laboratory, where they spent the night in the presence of a technician in order to ensure reliable control of the fasting. The following morning the basal metabolism tests were commenced at 7:00 A.M., following 30 minutes' rest in the basal metabolism bed after the body weight and height had been recorded. The number of hours' fasting, as well as the oral temperature, pulse rate, and blood pressure were recorded prior to the test. A 5-hour urine sample, starting at 7:00 A.M. was collected for the determination of nitrogen elimination. The greatest possible care was taken in handling of the subjects to achieve maximum relaxation.

It is hardly fair to compare the metabolism of Eskimos on high protein diets with White people on comparatively low protein diets, and perhaps one would be justified in speaking of an "Eskimo basal condition." In most of the previous studies the Eskimo subjects apparently were not basal, and our subjects were not strictly basal until the third day on the White man's diet. However, since we have determined the metabolism 14–18 hours after the last meal, this would

normally fulfill the requirement for "standard metabolism," "post absorptive metabolism" or "basal metabolism."

RESULTS AND DISCUSSION

In agreement with previous workers, it was found that the basal metabolism of Eskimos in their native habitat was significantly higher than in Whites, when examined for the first time while living on their own native diet. The average of the first test of all Eskimos of both sexes from all 4 settlements was 16% higher than the DuBois standard, and the women showed higher figures than the men. The highest figures were observed among the primitive Eskimos at Gambell and Anaktuvuk Pass, and the lowest figures obtained among the more civilized Eskimos at Kotzebue, where the average basal metabolism was only 5% higher than the DuBois standard. In 28 White men, 20–40 years old, examined for the first time, the BMR averaged only 2% below the DuBois standard, i.e., 18% lower than that of the Eskimos.

After having verified the results of previous investigators, the next problem was to explain the reason for this higher basal heat production in the Eskimo.

Of the various factors involved, apprehension would be expected to play an important part in the high basal metabolic rates observed in Eskimos when tested for the first time. It is quite difficult, however, to determine whether or not an Eskimo is apprehensive. The pulse rate and blood pressure cannot always be taken as an indication of the state of relaxation in the Eskimo, since his blood pressure and pulse rate normally are considerably lower than those of the Whites.

During our initial experiments, we observed in several cases a reduction of over 20% in the BMR when the same subject was tested repeatedly on successive days while living on the same diet, although the lowest BMR was still higher than in the White controls. The original high level was interpreted as evidence of apprehension. In some of the subjects, the basal metabolic rate has been determined on as many as 12 differ-

ent occasions, and we find that the drop in BMR generally levels out by the third test.

When comparing the basal metabolic rates in all of the Eskimo subjects as determined on the basis of the first test with the final test, when the lower level had been established on the third and subsequent days, we find an average reduction of 9% which is ascribed to the reduction in tension. This relative reduction was higher in women than in men, and was most pronounced in the most primitive groups.

It was observed that the Eskimo groups that had the highest basal metabolic rates also had the highest protein intake, and

TABLE 1

Average basal metabolic rate, protein intake and urinary nitrogen elimination in Eskimos

LOCATION	BASAL METABOLIC RATE		PROTEIN INTAKE	FASTING URINARY NITROGEN
	Cal./m²/hour	Deviation from DuBois standard		
		%	*gm/day*	*gm/hour*
Anaktuvuk Pass	46.3	+ 16	202	0.9
Gambell	42.9	+ 10	132	0.8
Barter Island	39.7	+ 2	129	0.6
Kotzebue	40.2	+ 3	98	0.6

when comparing the BMR with the urinary nitrogen elimination, the same positive correlation was observed. The highest BMR's and nitrogen eliminations were observed at Anaktuvuk Pass among the caribou meat eaters where the fasting urinary nitrogen at times was as high as 3 gm per hour. The relation between the average BMR, the average daily protein intake at the 4 Eskimo settlements, and the urinary nitrogen elimination determined during a 5-hour period, is shown in table 1.

It was also observed that the basal metabolism was higher in the winter when the protein intake was increased, than in the summer. At Gambell, for instance, the average daily protein intake, as well as the urinary nitrogen elimination, was 20%

higher in March than it was in August. In March the basal metabolic rate was 11% higher than the DuBois standard, while in August it was only 6% over the DuBois standard.

The relation between the high basal metabolism in the Eskimo and the diet has been suggested long ago by several workers, such as Heinbecker ('31), and later Höygaard ('41). Heinbecker studied the basal metabolism of Eskimos at Baffin Island in 1926 and again in 1930. During his first visit he found abnormally high figures for the basal metabolism associated with a high nitrogen metabolism, while during his second visit the basal metabolism was more than 20% lower, and the nitrogen metabolism was markedly reduced.

On the other hand, Odin ('37), who had observed that while basal metabolism was below DuBois standard in the Whites from Norrbotten in Northern Sweden, found that a significant rise in the basal metabolism occurred after an addition of 120–150 gm protein daily in the diet. Similar observations have been made by McClellan and collaborators ('31) and others.

It is well known that considerably higher amounts of protein are regularly consumed by the Eskimos (DuBois, '28), who generally speaking, prefer a diet where approximately 50% of the calories come from protein and the greater part of the remaining 50% are derived from fat. August and Marie Krogh ('13) report that the normal diet of the West Greenland Eskimos contained an excessive amount of animal protein — 280 gm daily — and they noted that there seemed to be a considerable delay in the metabolism of protein and excretion of nitrogen, only 60% of the nitrogen being excreted during the first 24 hours after eating large meals rich in protein. In East Greenland the Eskimos consume an average of 300 gm of protein daily (Höygaard, '41). In Alaska a daily protein consumption of more than 300 gm has been observed among the most primitive Eskimos.

In order to study the significance of the specific dynamic action of the protein in the high meat diet, with reference to the high basal metabolic rates in Eskimos, a special series

of studies was carried out on representative subjects from each of the 4 groups. These subjects, 14 men in all, were first studied in the field on their normal native diet, and then brought into our laboratory at Ladd Field where the basal metabolism again was tested under carefully controlled conditions on three or more different days while the subjects were eating the normal White man's diet. We then found that in all cases the basal metabolic rates were reduced to the range

TABLE 2

Basal metabolic rates in per cent of DuBois standard in 14 Eskimo men

LOCATION	SUBJECT NO.	ON NATIVE DIET	ON WHITE MAN'S DIET
		%	%
Gambell	1	+ 11	— 6
	2	+ 1	— 3
	3	+ 1	— 9
	4	+ 10	— 3
Barter Island	5	+ 9	— 10
	6	+ 14	— 4
	7	+ 13	— 4
	8	+ 6	— 14
Kotzebue	9	— 16	— 14
	10	+ 8	— 2
	11	+ 7	— 15
	12	— 6	— 13
Anaktuvuk Pass	13	+ 14	— 1
	14	+ 12	— 9
	Average	+ 8	— 8

of the normal White controls eating the same diet and examined under similar conditions (table 2). At the same time, the urinary nitrogen elimination was reduced to the same level as that of the White controls. Thus, when examined in their native habitat, the average BMR on the third and subsequent days of all the 14 Eskimo subjects was 8% higher than the DuBois standard, and the average urinary nitrogen was 1.2 gm per hour in these Eskimos. The average BMR in the same Eskimos when living on the White man's diet at Ladd

Field was 8% lower than the DuBois standard, and the urinary nitrogen was reduced to 0.4 gm per hour. This change occurred at the end of three days. In a group of 4 normal White men of similar age examined on three successive days under similar conditions and on the same diet, the final BMR was the same as in the Eskimos: 8% lower than the DuBois standard, and their urinary nitrogen elimination was the same as that of the Eskimos (table 3). Similar findings were made in another group of 12 White men examined later. It may be added that normal White controls examined by Dr. E. F. DuBois in New York (personal communication) excreted about

TABLE 3

Average basal metabolism, protein intake and urinary nitrogen elimination of normal white controls compared with Eskimos on White man's diet

SUBJECTS	BASAL METABOLIC RATE		PROTEIN INTAKE	FASTING URINARY NITROGEN
	Cal./m²/hour	Deviation from DuBois standard		
		%	*gm/day*	*gm/hour*
Normal White controls (♂)				
First test	40.4	— 2	75	0.4
Second test	38.9	— 6	70	0.5
Third test	37.8	— 8	60	0.4
Eskimos (♂) on White man's diet	37.4	— 8	73	0.4

0.5 gm urinary nitrogen per hour 14 to 18 hours after the last meal. It should also be noted that the so-called DuBois standards are generally recognized as being 6 to 8% too high, and that the original level, published in 1916, included the factor of tension in patients undergoing their first or second test.

In a separate experiment, the Gambell group was given a high meat diet, and their basal metabolism increased to 12% over the DuBois standard. A similar experiment, using normal White subjects on a high meat diet similar to that of the Eskimos, failed because of the fact that it was impossible for the subjects to become accustomed to eating such

great quantities of meat within the short time available for the experiment. In a later experiment, however, a group of 5 normal White men consumed 500 gm of meat daily (137 gm protein or 21.9 gm nitrogen), and an increase of 13% in the BMR was observed.

It is interesting to note that an Eskimo soldier who had lived for several months on the normal mess hall diet, had a basal metabolic rate 4% lower than the DuBois standard. It should also be noted that the above reported findings were essentially the same in both the 67 full-blooded Eskimos and in the 6 half-breeds examined.

Twenty R.Q. determinations were made in a group of 5 Eskimo subjects living on their native diet. The average R.Q. was 0.82, which is the figure upon which the calculations are based.

It has previously been suggested that the DuBois height-weight formula, when utilized for the calculation of body surface area, may not be applicable for the Eskimo, thus introducing an error in the calculation of the results, which might partly explain the high basal metabolic rates of the Eskimo. The body surface area was determined by the linear method in 53 of our Eskimo subjects, and it was found that the height-weight formula on an average gave results which were 0.9% higher than the results obtained by the linear method. This is within the limit of accuracy claimed for the height-weight chart (DuBois and DuBois, '16) and is therefore insignificant.

SUMMARY AND CONCLUSIONS

Three hundred and forty basal metabolism tests have been made on 73 healthy Eskimos from 4 different locations in Alaska, together with environmental observations, body surface area measurements, and determination of protein intake and nitrogen elimination. In agreement with previous workers, the basal metabolism of Eskimos examined for the first time in their native habitat was significantly higher than in the Whites. Approximately 9% of this higher basal metabolism may be accounted for by apprehension, and the high protein

Eskimo diet accounts for approximately 15%. When these two factors were eliminated, the metabolism was almost exactly the same as in the White controls.

From these studies it is therefore concluded that there are no racial differences between the Eskimos and the Whites in basal heat production.

ACKNOWLEDGMENTS

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FATE OF LYCOPENE IN THE RAT AND ITS EFFECTS ON THE UTILIZATION OF CAROTENE AND VITAMIN A [1]

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The effects of some structurally related compounds on the utilization of carotene and vitamin A have been reported previously (High and Day, '51). Due to the similarity between lycopene and carotene and the rather frequent occurrence of these substances together in nature, it was of interest to investigate the effects of lycopene on the utilization of carotene and vitamin A. Aside from experiments concerned with lycopene being a possible provitamin A (Drummond et al., '25) there are scarcely any data on the metabolic effects and the fate of the substance in the rat.

MATERIALS AND METHODS

The procedure employed for the preparation of lycopene was substantially the same as that described by Sandoval and Zechmeister ('49) except that purification by chromatography was omitted. Microscopic examination of the crystalline prod-

[1] Contribution 555 from the Department of Chemistry, Indiana University, Bloomington. Supported in part by grants from the Nutrition Foundation, Inc., and the Research Funds of the Graduate School of Indiana University. Some of the data are taken from a thesis submitted by Edward G. High to the Faculty of the Graduate School in partial fulfillment of the requirements for the Ph.D. degree, December, 1949. Presented in part at the 117th Meeting of the American Chemical Society in Houston, Texas, March, 1950.

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uct revealed an apparently homogeneous material consisting of long red needles. In hexane the absorption maxima were 445, 472, and 503 mμ and the absorption minima were 455 and 490 mμ. These data agree with both the absorption maxima and minima for pure lycopene in hexane (Zechmeister et al., '43). The molar extinction coefficient in hexane at 472–3 mμ was 16.9×10^4 and, employing the highest molar extinction coefficient reported of 18.6×10^4 (Zechmeister et al., '43), the product on analysis proved to be well over 90% pure lycopene.

Beta-carotene,[3] vitamin A,[4] and lycopene were dissolved separately in cottonseed (Wesson) oil in substantially the same manner as described by High and Day ('51). The depletion of vitamin A stores and the supplementation of the rats were accomplished in essentially the same manner as previously indicated (Kelley and Day, '50). All of the supplements were kept separate and stored under nitrogen in low actinic flasks at 4°C. and each was administered separately in 0.2 ml of cottonseed oil per rat per day.

Approximately 16 hours after the administration of the last supplement, each rat was sacrificed with ether. The livers and kidneys were removed promptly and analyzed immediately for both lycopene and vitamin A. Because lycopene was not detected in the kidneys, analysis of this organ for vitamin A was carried out as previously described (Kelley and Day, '48), by the colorimetric method of Sobel and Werbin ('46). The procedure for analysis of the liver for both vitamin A and lycopene was adapted from the method of Sobel and Werbin ('45), and the method of Dann and Evelyn ('38) was employed for carotene interference in the determination of vitamin A.

The livers were homogenized, saponified, and extracted with ether (Kelley and Day, '48). After removing the ether

[3] Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio, consisting of 85% β- and 15% α-carotene.

[4] Containing approximately 200,000 U.S.P units per gram. Kindly supplied by Distillation Products, Inc., Rochester, N. Y.

under vacuum the residues were dissolved separately in chloroform and initially analyzed for lycopene with a Klett-Summerson colorimeter employing a 440 mμ filter. The quantity of lycopene present was determined by reference to a standard curve for chloroform solutions of lycopene. Finally, these solutions were analyzed for vitamin A by adding separately 2 ml aliquots of each solution to 4 ml of activated glycerol dichlorohydrin and measuring the intensities of the colors developed at 6 minutes with a Klett-Summerson colorimeter employing a 540 mμ filter. The readings which were obtained represented both lycopene and vitamin A. The increments of these values due to the quantity of lycopene, as initially determined, were ascertained by reference to a standard curve for lycopene and the reagent. These increments were subtracted from the totals and the values for the differences were converted into micrograms of vitamin A by reference to a standard curve for vitamin A and the reagent.

When measured by means of a 540-mμ filter, the colors produced with both lycopene and the reagent, and vitamin A and the reagent, were additive and moderately stable at 6 minutes after mixing. Under these conditions 1.00 μg of lycopene was equivalent to approximately 0.14 μg of vitamin A. This is approximately the same relationship that exists between beta-carotene and vitamin A with activated glycerol dichlorohydrin as the chromogenic agent (Sobel and Werbin, '45).

RESULTS

Effects of lycopene in different amounts on the utilization of carotene and vitamin A

Two series of experiments were conducted to determine the effects of different amounts of lycopene on tissue deposition of vitamin A from supplements of carotene or vitamin A. In one series, each rat in a group received 30 μg of carotene dissolved in 0.2 ml of cottonseed oil per day for 12 days. A second group was treated similarly except that in addition

to the carotene, each rat was given 25 μg of lycopene per day. In a third group the amount of lycopene was 500 μg per rat per day. This was in addition to the carotene. The results are summarized in table 1. Twenty-five micrograms of lycopene significantly increased the hepatic storage of vitamin A by 53% while 500 μg were ineffective. The lycopene seemed to have no effect on the deposition of vitamin A in the kidneys or on growth.

TABLE 1

Effects of different amounts of lycopene on the utilization of carotene and vitamin A

DAILY SUPPLEMENT [1]	NO. AND SEX OF RATS	WEIGHT GAIN	VITAMIN A DEPOSITED		
			Liver	Kidneys	Total
		gm	μg	μg	μg
		Series 1			
30 μg carotene	8(2M)	26	18.4 ± 2.8	5.9	24.3 ± 3.0 [2]
30 μg carotene + 25 μg lycopene	8(2M)	26	28.2 ± 3.6	6.5	34.8 ± 2.4
30 μg carotene + 500 μg lycopene	10(3M)	23	19.1 ± 2.8	8.9	28.0 ± 2.7
		Series 2			
13 μg vitamin A	7(5M)	42	18.5 ± 1.4	9.5	28.0 ± 1.4
13 μg vitamin A + 25 μg lycopene	7(5M)	40	37.6 ± 7.9	10.1	47.7 ± 6.7
13 μg vitamin A + 100 μg lycopene	6(4M)	50	32.4 ± 6.5	8.3	40.7 ± 4.7
13 μg vitamin A + 500 μg lycopene	7(5M)	46	32.6 ± 7.4	13.3	45.9 ± 6.6

[1] Fed in cottonseed (Wesson) oil for 12 days.

[2] The standard error of the mean $= \sqrt{\frac{\Sigma(X-\bar{x})^2}{n(n-1)}}$ where X = individual values, $\bar{x}$ = the group mean, and n = the number of rats per group.

In another series (table 1) 13 μg of vitamin A were administered per rat per day in place of carotene. Rats that received 25, 100, or 500 μg of lycopene per day exhibited increases in hepatic storage of vitamin A as compared with the controls. However, there appeared to be no significant differences between the various levels of lycopene. The average increase in vitamin A deposition for the three groups

was 85%. No differences occurred between either the growth rate or the kidney vitamin A deposition in this series.

Hepatic lycopene deposition

The pigment in the liver of those rats that were fed lycopene with either carotene or vitamin A possessed absorption maxima in chloroform solutions of 457, 481, and 516 mμ. (Chloroform solutions of the unfed lycopene had similar absorption maxima.) According to Strain ('38), lycopene dissolved in chloroform possesses absorption maxima of 453, 480, and 517 mμ. Liver tissues of rats that received either carotene or vitamin A alone possessed negligible absorption in this region of the spectrum. Similarly, spectrophotometric analysis at 480 mμ for the quantity of lycopene present in the liver agreed with the colorimetric determination. From these observations, it seemed logical to conclude that the pigment present in the liver was lycopene. In no instance was the substance detected in the kidneys.

In order to gain information relative to factors influencing lycopene deposition in the liver, data were sought in regard to the deposition of the carotenoid as related to growth rate and to the quantity of lycopene fed. The animals that received 100 μg of lycopene with either carotene or vitamin A were divided into three groups on the basis of differences in weight gains. Both the lycopene deposition and the weight gain values were averaged for each group. The data are summarized in table 2. They indicated that the amount of lycopene deposited is inversely proportional to the rate of growth.

Data summarized in table 1 (series 2) show the relationship between the amount of lycopene ingested and the quantity deposited in the liver. Although the amount of lycopene deposited was higher with increased intake of the carotenoid, the efficiency of storage decreased with increased intake. For example, rats that received 25 μg per day for 12 days deposited in the liver 6% of the total amount ingested, while those that received 100 and 500 μg deposited 3.9 and 2.1%,

respectively. As much as 2.2 mg of lycopene were found in the livers of rats that received 2 mg of the carotenoid per day for 60 days. These data suggest that rats possess a large capacity for the storage of lycopene in the liver.

TABLE 2

Relationship between growth and quantity of lycopene fed and the hepatic deposition of lycopene

SERIES	NO. AND SEX OF RATS	LYCOPENE FED DAILY [1]	AVERAGE WEIGHT GAIN	AVERAGE LYCOPENE DEPOSITED
		μg	*gm*	*μg*
1	6F	100	23	61.8
	4F	100	38	51.1
	6(5M)	100	56	36.1
2	8(1M)	25	31	21.1
	9(1M)	100	31	54.7
	9(1M)	500	22	148.0

[1] Fed in cottonseed (Wesson) oil for 14 days.

DISCUSSION

The sparing effect of small amounts of lycopene on carotene and vitamin A may be due to protection of the provitamin and vitamin from oxidative decomposition in the alimentary tract as previously suggested for the effects of small amounts of lutein on carotene utilization (Sherman, '47; High and Day, '51). Its apparent failure to exert a protective effect on carotene when the amount ingested is large may be due to a type of competitive inhibition of the conversion of carotene to vitamin A. Thus, in the presence of large amounts of lycopene the sparing effect may be masked by an impairment in the formation of vitamin A. Some clarification of this problem may be achieved when it becomes possible to effect the enzymatic conversion of carotene to vitamin A in a strictly *in vitro* system.

Prior to this investigation scarcely any experiments had been reported concerning the fate of lycopene fed to rats. However, a few observations had been made on other species of animals. Gillam and Kon ('40) found no evidence of lyco-

pene absorption in cows. On the other hand, the carotenoid has been found in varying amounts in human abdominal fat, liver, and serum (Zechmeister and Tuzon, '35; Willstaedt and Lindquist, '36).

In contrast, the rat does not absorb appreciable amounts of either lutein or carotene as such. The factors responsible for this selective absorption of these carotenoids are obscure. Both lutein and carotene possess the ionone ring structure and lycopene is devoid of it. Possibly this difference in structure accounts for the difference in absorption.

SUMMARY

Relatively small amounts of lycopene fed with either carotene or vitamin A to vitamin A-depleted rats increased the utilization of both of the latter substances for tissue deposition of vitamin A. Large amounts of lycopene increased vitamin A deposition in vitamin A-fed rats but were without effect on vitamin A deposition from carotene. Lycopene was absorbed and deposited in the livers of rats in approximately 2–6% of the total amount ingested. No lycopene was detected in the kidneys.

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EVALUATION OF NITROGEN SOURCE MATERIALS BY AN INTRAPERITONEAL RAT TEST[1]

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ONE FIGURE

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The biological value of a nitrogen source material is an index of the efficiency with which it meets the nitrogen needs of an animal. Methods of estimating the biological value of protein hydrolysates and of amino acid mixtures intended for intravenous use in states of protein depletion currently are receiving a great deal of attention both by interested laboratories and by such regulatory agencies as the Food and Drug Administration and the Pharmacopoeial authorities. Methods are vitally needed for distinguishing between preparations which have little or no biological value, and those which have been formulated so as to meet adequately the needs of the organism.

Two methods currently being studied for official adoption are based either on intravenous administration into dogs (Frost and Risser, '46; Kade, Houston, Krauel and Sahyun, '46; Silber and Porter, '49) or on oral ingestion by rats (Frazier, Wissler, Steffee, Woolridge and Cannon, '47; Frost and Sandy, '48). The former procedure is not practical for routine use since it is time consuming and costly. Rat feeding methods should be objected to because they do not simulate the conditions of use, since they permit the intestinal enzymes to digest any contained peptides in a manner which

[1] Presented in part before the American Institute of Nutrition, Cleveland, April 30, 1951 (Arnold and Shepherd, '51).

does not take place after intravenous injection. As an example, the availability of the peptide nitrogen after oral ingestion by dogs of one preparation was indicated by Silber and Porter ('49) as the reason for its good biological value, although it had a value of zero when administered intravenously. Obviously, such a preparation would be of little value to a patient being fed by vein.

A third method, suggested by Silber and Porter ('50), depends upon determining the nitrogen retention of rats given a preparation parenterally at a single dosage level over a one- to 4-day period. Quantitative results in terms of biological value are obtained by the method of Allison and Anderson ('45), the endogenous nitrogen excretion being estimated by including a depletion control group.

In the present study it has been shown that when the test is set up at multiple levels of intake, the indicated nitrogen retention is markedly different from that estimated by Silber and Porter ('50) on the basis of a single level of intake and a depletion control group. Therefore, the results from a single dosage level experiment may not yield correct values. However, those based on multiple dosage levels obviate the need for a depletion control group, and are a valid index of the biological value of the test preparation. Data illustrating these points are given in the following paragraphs. Furthermore, if the method presented herein is generally adopted, it will afford a rapid and convenient method of measuring the biological value of intravenous protein injection mixtures, and will lend confidence to the selection of appropriate solutions for clinical use. In addition to the description of the method for evaluating nitrogen source materials given in the following sections, the results of applying the method to preparations currently employed clinically and to gelatin are included.

EXPERIMENTAL

Test materials

Defatted whole eggs. Vacuum packed dried whole eggs were extracted with hexane in a two-liter capacity Soxhlet extractor

for three days. The solvent was removed from the extracted eggs by allowing them to remain in thin layers on paper overnight.

Tryptophan-deficient hydrolysate. A tryptophan-free hydrolysate was prepared for these studies by Dr. A. Lesuk of this Institute who hydrolyzed casein with dilute sulfuric acid. In the absence of reducing agents, this procedure destroys tryptophan. The hydrolysate, after the removal of the acid with barium in the usual way and supplementation with small amounts of methionine and phenylalanine to compensate for losses during the treatments, was concentrated to a 6% solution of the hydrolyzed protein. The solution was sterilized for use and was supplied in 1 l portions.

Gelatin. To determine the effect of peptide-bound nitrogen under these conditions of test, a gelatin preparation was administered both orally and parenterally. The preparation used was a 6% one identified by the Knox No. P 48–20. It had not been prepared to serve as a source of nitrogen so much as for use as a plasma extender.

Commercial hydrolysates and amino acid mixtures. Five commercially available preparations intended for clinical use were evaluated by the proposed procedure. They were obtained on the market.

Diet

The essentially nitrogen-free diet had the following composition (in parts per 100): dextrin 41.88, glucose (cerelose) 41.87, salts (Jones and Foster, '42) 4, methyl cellulose 2, cod liver oil (2,000 units of vitamin A, 250 units vitamin D per gram) 1, hydrogenated vegetable oil (Primex) 9, choline chloride 0.15, inositol 0.1; (in milligrams per 100 gm) calcium pantothenate 2, riboflavin 0.6, niacinamide 0.4, pyridoxine hydrochloride 0.4, folacin 0.2, *p*-aminobenzoic acid 0.2, thiamine hydrochloride 0.2, menadione 0.02, biotin 0.02, and vitamin B_{12} 0.002.

Procedure

Female rats of 200 to 300 gm body weight were caged individually. To reduce their nitrogen stores they were fed the nitrogen-free diet ad libitum for a week. They were then fed weighed amounts of the diet each day over a 4-day period. The amount fed was one that all of the rats given any one nitrogen supplement would voluntarily consume, e.g., 8 gm per 200 gm rat per day. The diet was fed each morning before the supplements were administered.

The test materials were administered intraperitoneally using sterile precautions with needles of about 24 gauge and syringes of 5 to 15 ml capacity varying with the amount of solution given. The site of injection on the rats was swabbed with 70% alcohol prior to injection. In addition, to reduce the risk of infection, about 200,000 units of penicillin per liter were added to the solutions.

The animals were allowed one day for equilibration. Then, at the time they received their second injection, the rats were placed in metabolism cages. Aside from rinsing the sides of the cages with a little water each day the urine and feces samples required no other attention until they were collected at the end of the three-day experimental period. The possibility of nitrogen loss was minimized by adding a crystal of thymol and a few drops of sulfuric acid to the urine collecting bottles at the time they were placed under the funnels. The feces, kept separate from the urine by having been caught on glass wool at the neck of the metabolism rack funnels, were collected and placed in 1 : 1 sulfuric acid for stirring prior to taking aliquots for analysis.

Analyses

Analyses for nitrogen were made by the macro-Kjeldahl method, using copper as a catalyst. The food and feces nitrogen samples were digested for 6 hours after clearing and the urine samples for one and one-half hours.

TABLE 1

Biological value of defatted whole egg fed to one-week protein depleted adult rats and of a tryptophan-free hydrolysate administered intraperitoneally

TEST SUBSTANCE	NUMBER OF RATS	AVERAGE WEIGHT		NITROGEN INTAKE	NITROGEN EXCRETION		NITROGEN "BALANCE"		BIOLOGICAL VALUE	
		At depletion	Final		Urinary	Fecal	N intake − urinary N	N intake − (UN+FN)	Based on UN	Based on UN+FN
		gm	*gm*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*		
Test no. 16										
None	3	238	219	...	192	94	...	−286		
Defatted	5	234	222	145	182	95	−37	−132		
whole	6	230	218	200	179	106	+21	−85		
egg	5	223	214	289	176	113	+113	0	1.04	0.92
Test no. 17										
None	4	254	236	...	171	73				
Defatted	6	252	236	135	169	89	−34	−123		
whole	6	252	242	181	175	82	+6	−76		
egg	6	252	244	255	173	81	+82	+1	0.98	1.03
Test no. 18										
Tryptophan-free	6	284	270	149	205		−56			
hydrolysate	5	283	271	207	271		−64			
	6	284	269	299	355		−56		0.0	

TABLE 2

Biological value of a 6% gelatin solution (Knox no. P 48–20) given orally or intraperitoneally

ROUTE OF ADMINISTRATION	NO. OF RATS	AVERAGE WEIGHT		NITROGEN INTAKE	NITROGEN EXCRETION		NITROGEN BALANCE		BIOLOGICAL VALUE	
		At depletion	Final		Urinary	Fecal	UN only	UN + FN	UN only	UN + FN
		gm	*gm*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*		
Orally	6	238	227	226	177		48			
	5	240	229	321	272		48			
	5	239	232	461	337		124		0.15 ±	
	6	234	227	682	577		105		0.06	
Intra-peritoneally	6	216	206	76	201	62	−125	−187		
	6	216	205	150	266	52	−116	−168		
	6	216	207	298	386	56	−88	−144	0.18 ± 0.06	0.19

Calculation of results

The evaluations are based upon the slope of the regression line calculated from the data which show the nitrogen balance at different levels of nitrogen intake. The slope of this line is an expression of the rate of change of nitrogen balance with respect to nitrogen intake and is a valid measure of the biological value of the protein. It has been used by Bricker and Mitchell ('47) for evaluating proteins ingested by rats

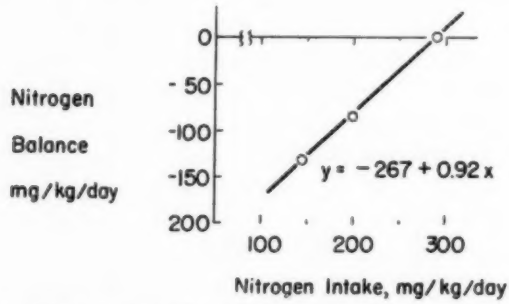

Fig. 1 Graphical representation of increase in nitrogen balance (retention) of rats fed defatted whole egg protein in increasing amounts.

orally, and by Melnick and Cowgill ('37) and Allison and Anderson ('45) for dogs given nitrogen source materials orally and parenterally.

The constant 'a' of the regression equation $y = a + bx$ (Waugh, '43) is the calculated nitrogen balance at zero nitrogen intake. For defatted whole egg of test no. 16 given in table 1 the value calculated for 'a' is — 267 mg nitrogen per kilogram per day. It may be compared with the experimentally determined value of — 286 mg nitrogen per kilogram per day given in table 1. The constant 'b' of the regression equation is the slope of the line. The data for defatted whole egg of table 1 are given in figure 1 to demonstrate representative results graphically.

RESULTS AND DISCUSSION

Defatted whole egg protein. It may be seen from the data summarized in table 1 that the nitrogen of defatted whole egg was essentially completely retained and the biological value of the protein was very close to 1.0. Since the defatted whole egg protein was fed along with the diet, the results do not bear upon the matter of parenteral feeding. It appears reasonable to conclude, however, that the data offer supporting evidence for the view that, aside from the matter of the parenteral administration of nitrogen source materials, which is discussed below, the over-all procedure might be expected to yield valid conclusions.

A separate point which may be seen from the feeding of defatted whole egg protein is that under conditions of controlled food intake the fecal nitrogens are relatively constant. This being the case essentially the same biological value is indicated from the calculations based upon urinary nitrogen alone or upon both urinary and fecal nitrogen excretions.

Tryptophan-deficient hydrolysate. The effect of administering a poor nitrogen source material upon nitrogen retention is presented in the data obtained by administering a tryptophan-free hydrolysate, as summarized in table 1. There was increased nitrogen excretion with increasing nitrogen administration so that the nitrogen retention did not increase. In accordance with the expected result, the biological value of the material was accordingly zero.

However, if the slope of response, e.g., the biological value, had been based upon the nitrogen excretion at zero nitrogen intake (171 to 192 mg/kg, table 1) and any one of the balance values observed for the tryptophan-free hydrolysate (— 56 to — 64 mg/kg), some positive value would have been ascribed to the preparation, which would have been erroneous. It is clear that misleading conclusions may be drawn from single level tests.

An instance of this in the literature may be used to emphasize the point. Silber and Porter ('50) ascribed positive biological value to amino acid mixtures deficient in one or another of the essential amino acids. Their calculations were based upon one of their test levels along with the nitrogen excretion at zero nitrogen intake. However, if the latter value is not used, and if their data are recalculated on the basis of the nitrogen balances at the two levels of administration, the evaluations from their data are in accord with the expected results, namely, amino acid mixtures deficient in one of the essential amino acids do not support nitrogen retention at increasing levels of nitrogen administration. Stated in another way, their preparations had biological values of essentially zero. Results such as these, in addition to our own observations given above, support the validity of conclusions based upon multiple level tests.

Gelatin. Because of the possible interference of peptide-bound nitrogen in test materials evaluated by the proposed procedure, a preliminary check on this effect was made by giving 6% gelatin (Knox No. P 48–20) orally and parenterally. The results are summarized in table 2. They do not supply any instance of higher nitrogen retention by oral than by parenteral administration, in spite of a probably high proportion of peptides. The value 0.15 to 0.19 for gelatin reported here may be compared with that of 27% nitrogen retention observed in dogs by Rhode, Parkins and Vars ('49) and that of 25% by Mitchell, Beadles and Kruger ('27). Rhode et al. ('49) noted considerable individual variation, which is also indicated in these studies by the high standard error of the results.

The 4-level oral assay of gelatin relates to the matter of straight line responses. Without discussing the data in detail, it is apparent from the nitrogen balance data of table 2 that so much variation existed that almost any value for gelatin could have resulted if less than 4 levels of intake had been given depending on which, and how many, of the points had been omitted. Whether the reason for the wide variance

in data yielded in the tests on gelatin is other than its high content of peptides is not evident to us. In any event, gelatin does not meet the biological value criterion of 0.40, i.e., 40% nitrogen retention, suggested by Mitchell ('52) as the minimum necessary to support growth in some species. Mitchell's suggested criterion of biological value is met by each of the commercial hydrolysates discussed below, which clinical experience has shown to be useful. Thus, Mitchell's further suggestion that "biologic evaluation of proteins and protein mixtures is still the 'court of last resort'" is fully supported by the findings submitted here.

As a separate point, it may be seen from the data in tables 1 and 2 that the rats lost weight during the test. This was due to the fact that the voluntary food intake of the animals decreased during the depletion period to a point where they would not voluntarily consume their caloric needs during the test (repletion) period. The loss in weight did not vary widely for the animals administered any one preparation and the results did not depart from linearity to a significant extent. This may have been due to the short period of time of the test period; continuing weight loss over a longer period of time might have exerted a discernible unfavorable effect. We have not studied this point, however.

Commercial hydrolysates and amino acid mixtures. The results of applying the proposed procedure to commercially available nitrogen source materials intended for parenteral use are summarized in table 3. It may be seen that the preparations have biological values in the range of 0.44 to 0.63, when the evaluations are based on total nitrogen excretion. The values obtained by this procedure are in the same range as those reported by Silber and Porter ('49) for comparable preparations evaluated by parenteral administration in dogs. Tested orally in dogs the same investigators noted that the nitrogen of the preparations was retained somewhat better, 61 to 73%.

It is seen that all of the preparations tested here were well utilized. This is in contrast to the report of Silber and Por-

ter ('49). These investigators noted a preparation (C) which was well utilized when given orally (66 ± 2.0% nitrogen retention) but which was not utilized at all parenterally (0 ± 13%). Presumably, this occurred because the digestive enzymes rendered available one or more peptide-bound essential

TABLE 3

Biological value of commercially available nitrogen source materials intended for intravenous alimentation evaluated by the multiple level parenteral rat test

PREPARATION	TEST NO.	NUMBER OF RATS	NUMBER OF LEVELS OF TEST	BIOLOGICAL VALUE BASED ON	
				UN + FN	UN only [1]
A	21	12	3	0.495	0.61 (± 0.09) r = 0.91 [2]
	33	18	2	0.51	0.58
B	22	13	3	0.63	0.56 (± 0.12) r = 0.82
	25	12	3	0.57	0.54 (± 0.09) r = 0.89
C	24	14	4	0.50	0.51 (± 0.07) r = 0.90
	23	14	3	0.48	0.53 (± 0.15) r = 0.71
D	25	10	3	0.62	0.62 (± 0.11) r = 0.90
	27	15	3	0.49	0.52 (± 0.11) r = 0.80
E	27	15	3	0.44	0.47 (± 0.11) r = 0.96
	28	15	2	0.48	0.49 (± 0.08)

[1] Along with the biological value is given its estimated standard error where (Snedecor, '46) $s_b = \sqrt{(Sy^2 - (Sxy)^2/Sx^2)/(n-2)\ (Sx^2)}$.

[2] The correlation coefficient, r, was calculated from the Waugh ('43) formula $r = Sxy/(Sx^2)\ (Sy^2)$.

amino acids which were not available when the preparation was given parenterally and were not acted upon by the digestive proteolytic enzymes. The question of the effect of the route of administration thus does not appear to arise when the proposed procedure is applied.

Several additional points of importance may be discussed on the basis of the data supplied from the study of the clinical preparations. More often than not, the evaluation based solely on urinary nitrogen excretion agrees with the evaluation based on total excretion, but the instances of disagreement which do occur indicate that misleading conclusions might be drawn from time to time if the fecal nitrogen output is not taken into account. Thus, we cannot recommend that determination of the fecal nitrogen excretion be omitted.

It has been our practice to examine the individual urines in preference to a pooled sample in order to obtain some indication of the error of procedure. The fecal excretions take more time to prepare so that on these we have only analyzed the pooled samples. Therefore, the balance values based on urinary plus fecal nitrogen excretions are not characterized by an estimate of the error (s_b).

Also, it may be noted from the data in table 3 that the preparations have been tested at two, three, or 4 levels of intake. The r (correlation coefficient) values indicate that the data satisfy the statistical criteria for a straight line response. This correlation coefficient is meaningful only when the preparations are tested at three or more levels. Having established this point, a two-level test with the nitrogen intakes widely spaced, e.g., 100 and 300 mg/kg, favors reducing the error of the estimation (Smith, '51).

At this time, on the basis of the data submitted above, the proposed procedure thus appears to offer a valid method of evaluating nitrogen source materials parenterally in a simple manner.

SUMMARY

A method is described whereby nitrogen source materials intended for intravenous alimentation may be evaluated by intraperitoneal administration to adult rats, which have been protein depleted for one week. In its simplest form, the preparations are administered at two levels for 4 days, excreta collections being made during the last three days. The excreta may be pooled for analysis.

Evaluation of a 6% gelatin solution by both parenteral and oral test indicated it to have about the same biological value, 0.15 to 0.19, thus leaving open the question as to the nutritive value of the contained peptide-bound amino acids.

Five commercial preparations of known clinical usefulness when parenterally administered were observed to have biological values in the range of 0.44 to 0.63.

The data show conclusively that evaluations based upon multiple levels of test may be materially different from those based upon single level tests in conjunction with the nitrogen excretion at zero nitrogen intake. The use of multiple levels thus appears to be mandatory for valid assays.

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NITROGEN BALANCE OF ADULT RATS FED DIETS LOW IN L- AND DL-LYSINE, OR DEVOID OF ARGININE [1]

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ONE FIGURE

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The indispensability of lysine for rat growth has been firmly established. Most investigators are convinced that lysine is also indispensable for the maintenance of nitrogen or weight equilibrium in the adult animal. Wolf and Corley ('39) found that omission of any one of the essential amino acids, except arginine, from the diet of adult rats resulted in negative nitrogen balance and weight loss. Frazier et al. ('47) also observed weight losses under similar experimental conditions. The adverse effect of withdrawal of lysine on nitrogen balance was confirmed by Wissler et al. ('48). Gillespie et al. ('45) reported that rats deprived of dietary lysine developed a mild anemia. They suggested, however, that this response was not specific for lysine deficiency and that it might occur in the absence of any of the other essential amino acids. Rose and Rice ('39) demonstrated that the adult dog requires dietary lysine for the maintenance of nitrogen equilibrium. The minimum quantity of lysine required by the adult rat for the maintenance of body weight was estimated by Neuberger and Webster ('45) to be 16 mg/day/rat (three animals with average body weight of 335 gm),

[1] This investigation was supported in part by a grant from the Office of Naval Research, NR contract N6ori-126 Task IX.

and by Steffee et al. ('50) to be 60 mg/day/kg (150-gm rat). Benditt et al. ('50) reported that the minimum daily requirement of lysine for maintenance of nitrogen equilibrium in the adult male rat is 3.7 mg/day/100 cm^2 of body surface.

Burroughs, Burroughs and Mitchell ('40) concluded that the adult rat does not need lysine, leucine, histidine, phenylalanine or arginine for the replacement of endogenous losses of nitrogen if threonine, isoleucine, tryptophan, valine, methionine, tyrosine and norleucine are present in the diet. Mitchell ('47) reiterated this point of view with respect to lysine and concluded that ". . . lysine is entirely dispensable in adult rodent nutrition, or is required in inconspicuous proportions for the maintenance of nitrogen equilibrium."

The experiments on lyine described below represent a portion of a larger project which eventually will provide quantitative data on the requirement of the adult rat, under uniform conditions, for all of the essential amino acids.

METHODS

Adult male albino rats of the Wistar strain were used. At the beginning, and after a 48-hour fast with water ad libitum, the body weights ranged from 210 gm to 254 gm (mean = 232 gm). The energy allowance for each rat was 40.4 Cal./day. Many observations in this laboratory make it evident that energy and weight balance are satisfactorily maintained if 121 Cal./day are allowed for each unit of metabolic body size, i.e., Cal./rat/day = 121 $kg^{3/4}$. Each experiment included the following sequence of feedings: maintenance diet (9.6% whole egg protein), two weeks; nitrogen-free (N-free) diet, one week; amino acid diet, containing approximately half of the maintenance requirement of total nitrogen (half-N), one week; amino acid diet, containing twice this amount of total nitrogen (full-N), one week. This 5-week cycle of feeding was repeated for each amino acid mixture investigated.

The amino acid mixture simulates the proteins of whole egg in that it contains approximately the same amount of each natural isomer of the essential amino acids per gram

of total nitrogen. The non-essential amino acids of egg protein are replaced, in this mixture, by the unnatural isomers of 6 essential amino acids plus sufficient L-glutamic acid to make up the same amount of total nitrogen. This "complete" amino acid mixture and the basal diet in which it is used are described elsewhere (Anderson and Nasset, '48; Nasset, Anderson and Siliciano, '51).

The N-free diet was compounded simply by omitting the amino acid mixture and substituting an equal weight of sucrose.

It is the custom in this laboratory to observe changes in nitrogen balance which are brought about by a stepwise reduction of the concentration of one essential amino acid at a time. This process simulates the consecutive testing of a series of proteins of different biological values. It is probably a much more physiological procedure than omitting completely one of the essential amino acids from the mixture. At some point in this stepwise reduction a concentration is reached which becomes limiting for the utilization of the total dietary nitrogen. In the present investigation two-fifths and one-twenty-fifth L-lysine and one-twelfth DL-lysine amino acid mixtures were used. In addition, one mixture was tested in which arginine was absent. The fractions refer to the total amounts of lysine present, regardless of isomeric form, as compared with the "complete" amino acid mixture. One-twelfth DL-lysine, for example, simply refers to a mixture which contains one-twelfth as much lysine nitrogen as the complete mixture and identifies the source as the racemic form.

The N-free, half-N and full-N diets were fed by stomach tube in two equal portions daily. The maintenance diet was weighed out accurately each day in feeding cups to provide each animal with a constant intake of protein and energy. This portion was almost invariably eagerly and completely consumed. Total N was determined on feed and excreta by the Kjeldahl method. Each diet period lasted 7 days and feces were collected for the entire period. They were marked

by adding Cr_2O_3 to the first feeding of each period. The urine was collected for analysis only during the last 4 days of each period of amino acid feeding. Other details of experimental procedure are described in an earlier paper (Nasset and Anderson, '51).

RESULTS

Table 1 contains the average results of all experiments with the rats of series 320. The discovery of a limiting concentration of the essential amino acid being studied may require considerable preliminary work. The first guess is usually made on the basis of data taken from the literature but this technique invariably yields estimates which are too high. Inspection of the results of experiment II shows at once that the nitrogen balance, the value of K′ and the total nitrogen computed to be necessary for the maintenance of nitrogen equilibrium (NI_e) are unaffected by reducing the L-lysine content of the diet to two-fifths of the lysine content of the complete diet (experiments I, V and VI). According to these three criteria the amino acid mixture used in experiment II is nutritionally equal to the one used in experiment I. According to the same criteria the one-twenty-fifth L-lysine amino acid mixture is significantly poorer than the ones used in experiments I and II and here the small amount of L-lysine available is affecting adversely the utilization of the dietary nitrogen. Fortunately then, a limiting concentration of L-lysine which was not too severely deficient was discovered on the second trial.

The responses to feeding the complete amino acid mixture, as well as those in which the amino acid being investigated has not been critically reduced (experiments I, II, V and VI), confirm many previous experiments in this laboratory. A conspicuous feature is the absence of any change in either fecal or urinary N excretion when the intake of N is doubled. This fact indicates complete utilization of the increment in N intake and speaks well for the adequacy of the complete amino acid mixture for the maintenance of N equilibrium

TABLE 1

Average data for adult rats receiving amino acid diets

EXPERIMENT NUMBER	SERIES 320					
	I	II	III	IV	V	VI
AMINO ACID MIXTURE	Complete	2/5 L-Lysine	1/25 L-Lysine	1/12 DL-Lysine	Complete	Complete minus arginine
Number of rats	11	14	13	10	11	10
Average body weight (kg)	0.240	0.246	0.244	0.253	0.261	0.269
Metabolic body size ($kg^{3/4}$)	0.343	0.349	0.347	0.357	0.365	0.374
	Milligrams of nitrogen per day per $kg^{3/4}$					
Half N-period:						
N intake	70	71	72	67	69	65
Fecal N	37	34	33	31	30	28
Urinary N	109	108	121	101	102	105
N balance	−76	−72	−82	−66	−63	−68
Full N-period:						
N intake	140	136	141	132	132	129
Fecal N	34	31	31	29	26	25
Urinary N	109	110	146	134	112	105
N balance	− 4	− 5	−35	−31	− 7	− 2
K′ (half-N; full-N)[1]	1.03 ± 0.06	1.01 ± 0.04	0.67 ± 0.03	0.53 ± 0.04	0.90 ± 0.03	1.06 ± 0.06
NI_e[2]	141 ± 4	141 ± 2	195 ± 4	194 ± 7	140 ± 3	130 ± 3

[1] K′ is the slope of the line joining the half-N and full-N points when nitrogen balance is plotted against nitrogen intake.
[2] NI_e is the nitrogen intake computed to be necessary for attainment of nitrogen equilibrium.

under these experimental conditions. For the reader who is unaccustomed to the unit of metabolic body size ($kg^{3/4}$), it may be helpful to state that the total N intake of the average 250-gm rat on a full-N diet was approximately 50 mg/day.

DISCUSSION

The results in table 1 reveal that a rather drastic reduction in lysine intake is required to produce an adverse effect on nitrogen balance in the adult animal. In experiment II (two-fifths L-lysine) the lysine was reduced to 40% of the amount in the complete amino acid mixture (experiments I and V) without causing any detectable change in nitrogen balance. In fact, the values for nitrogen balance, K′ and NI_e, are virtually identical in experiments I and II. When the concentration of lysine is reduced to 4% (one-twenty-fifth L-lysine, experiment III), however, the changes, when compared with experiment II, are significant throughout. Nitrogen balance is depressed at both levels of intake ($P<0.03$ and <0.0001 for half-N and full-N, respectively), K′ is reduced ($P<0.0001$) and the amount of nitrogen computed to be required for nitrogen equilibrium, NI_e, is increased ($P < 0.0001$).

The preponderance of evidence supports the view that D-lysine is not utilized by the animal for either growth or maintenance. McGinty et al. ('24) reported that racemic lysine was only half as effective as the natural isomer in promoting growth of rats on a diet containing gliadin as the source of protein. Berg ('36) observed that the growing rat is unable to utilize D-lysine as a supplement to zein and Totter and Berg ('39) made a similar observation on the young mouse. Kratzer ('50) demonstrated that turkey poults are unable to utilize D-lysine for either growth or feather pigmentation. Rose ('49) reported, without supporting data, that D-lysine cannot be utilized by the adult human male. Albanese ('47) stated, on the basis of unpublished results, that more than 80% of orally administered D-lysine is utilized by man.

The results of the present investigation show conclusively that the adult rat is unable to utilize D-lysine in the mainte-

nance of nitrogen balance. A comparison of the results of experiments III and IV (table 1) shows clearly that one-twelfth DL-lysine is approximately equivalent to one-twenty-fifth L-lysine with respect to nitrogen balance, K′ and NI_e. Such a result would be expected if the unnatural component of the racemic mixture were inactive.

Melnick and Cowgill ('37) and Bricker et al. ('45) demonstrated in dogs and humans, respectively, that nitrogen balance is a linear function of protein intake, especially when nitrogen balance is negative. In previous work with valine, methionine and threonine (Nasset and Anderson, '50, '51; Nasset, Anderson and Siliciano, '51) it was demonstrated

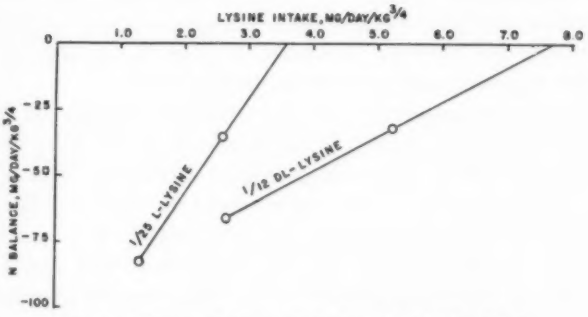

Fig. 1 Nitrogen balance plotted against lysine intake.

that nitrogen balance is a linear function of the intake of the limiting essential amino acid. This fact was used to compute the daily requirement of these amino acids for the maintenance of N equilibrium.

In figure 1 nitrogen balance is plotted against lysine intake for the two experiments in which this amino acid was present in limiting concentrations (experiments III and IV, table 1). An extrapolation upward of the line joining the two average points obtained from each of these experiments yields graphic estimates of the lysine required for maintenance of N equilibrium. A more satisfactory estimate is obtained by extrapolating the lines joining the individual points

obtained for each rat for each experiment. The mean values thus obtained, expressed as milligrams of lysine/day/kg$^{3/4}$ required for nitrogen equilibrium are: L-lysine = 3.6 ± 0.07 and DL-lysine = 7.8 ± 0.27. The results demonstrate clearly that D-lysine is not utilized under these conditions. Using the methods of Nasset and Siliciano ('52), the results of Benditt et al. ('50) and Rose ('49), for the adult rat and human, respectively, were computed on the same basis. The requirement of lysine as determined by these investigators is roughly 10 times as great as the value reported here. Benditt et al. ('50) used very few animals in their work and this may account for their relatively high value. An obvious reason for the high value given by Rose is the fact that what he reports as a minimum requirement is not a mean of his minima but actually his highest minimum among the group of subjects who ingested any particular amino acid.

The evidence presented for the indispensability of lysine in the nutrition of the adult rat appears to be unequivocal. The requirement is very small under these conditions, i.e., 3.6 mg lysine/day/kg$^{3/4}$, or approximately 1.25 mg/day/250-gm rat. This fact may account for Mitchell's ('47) conclusion that lysine is dispensable for the adult rat. He fed 4 rats 10 gm/day of a diet in which the protein was chiefly derived from white flour and the total daily nitrogen intake for each rat was 67.6 mg. If it is assumed that all of the nitrogen came from white flour, it can be computed from the data of Block and Bolling ('51) that Mitchell's rats may have been ingesting approximately 8 mg of lysine per day. Since this is several times the minimum requirement, as established in the present investigation, it is to be expected that supplementation of white flour with lysine would fail to improve the nitrogen balance.

SUMMARY

Nitrogen balance was determined on a group of adult male albino rats which derived all of their dietary nitrogen from mixtures of amino acids. Each experiment included the following dietary regimens in the order given: 14 days on mainte-

nance diet (9.6% whole egg protein); 7 days on N-free diet; 7 days on amino acid diet supplying approximately half of the maintenance requirement of total nitrogen; 7 days on double the quantity of the amino acid mixture fed in the previous period. These diets, except the maintenance diet, were fed by stomach tube in two equal portions daily, and each rat received the same amount of diet each day.

If the quantity of lysine in a complete mixture of essential amino acids is reduced sufficiently, the nitrogen balance is adversely affected. When this amino acid is the limiting factor in the utilization of dietary nitrogen, it is assumed that N balance is a linear function of lysine intake. On this assumption the requirement for the maintenance of N equilibrium is 3.6 ± 0.07 mg of L- and 7.8 ± 0.27 mg of DL-lysine/day/kg$^{3/4}$.

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EVALUATION OF THE BIOLOGICAL VALUES OF THE PROTEINS IN FISH MEALS BY THE NITROGEN RETENTION METHOD[1]

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A review of the literature shows the need of detailed information on the biological values of the proteins in fish. Deuel and associates ('46) found that mixed mackerel muscle proteins have a considerably higher biological value than casein. Tuna proteins when fed at 9 and 12% and sardine proteins at 15% also gave considerably more rapid growth in weanling rats than diets containing similar amounts of casein.

Since fish meals, which are by-products of the fish industry, are being used extensively as sources of proteins in animal feeding, it was thought of interest to investigate their biological values. With the cooperation of the By-Products Division of the National Fisheries Institute, Washington, D. C., we were supplied with the following products from various parts of the country: sardine meal; Alaska herring meal; herring meal with fish solubles, which is from a different source than the Alaska herring meal; Red fish meal; Menhaden fish meal; anchovies meal; and crab meal. The fish meals were furnished by the processors in 50-lb. lots selected from several batches; hence the products received constituted representative samples.

The biological values of the proteins in the fish meals were determined by the nitrogen balance method of Mitchell ('24,

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'44), in which the values are expressed as the per cent of absorbed nitrogen retained by the animal. From the biological values and digestibility figures the per cent of net utilization was calculated. Wistar strain albino rats were used and in all of the groups the sexes were divided equally. Each group contained 12 animals. The animals were about 28 days old when started on the experiments and they weighed 50 to 56 gm each. In order to eliminate the influence of the plane of nutrition, we used controlled feeding. Each group was fed the fish meals at an 8% protein level; each animal was given 8 gm daily of its ration which was totally consumed by every rat in the group; therefore, the food intake was constant. Each ration contained an amount of fish meal to provide 8% protein. The rest of the rations contained percentagely cellu flour for roughage, 2; Sure's salts No. 1 ('41), 4; vegetable shortening, 8; cod liver oil, 2; wheat germ oil, 1; and the balance, cerelose. The fat-soluble vitamins A, D, and E were furnished by the cod liver oil and wheat germ oils. The following components of the vitamin B complex were administered separately from the ration 6 times weekly: 25 μg of thiamine, riboflavin, pyridoxine, and niacin, respectively; 150 μg calcium pantothenate, 1 mg inositol, 3 mg *p*-aminobenzoic acid, and 9 mg choline chloride.

In order to have information on the biological values of the proteins in fish meals expressed in comparison with efficiency of proteins of known excellent quality, nitrogen retention studies were also carried out on dried whole eggs and on dried non-fat milk solids.

Urinary and fecal balances were carried out for 7 days on an egg standardizing ration and also for 7 days on the experimental rations containing the various fish meals. In each case the animals were allowed to become accustomed to consuming the entire rations for a pre-test period of from three to 5 days before the beginning of the nitrogen balance studies. The percentage composition of the egg ration is as follows: dried defatted whole egg, 5.8; cellu flour, 2.0; Sure's salts No. 1, 4; vegetable shortening, 8.0; cod liver oil, 2; wheat

germ oil, 1; and cerelose, 77.2. The defatted dried whole eggs contained 69.2% protein and furnished 4.0% protein in the ration.

The results of this study are summarized in tables 1 and 2. It will be noted from table 1 that the protein content of the fish meals varied from 35.0 to 69.7%; the fat from 2.1 to 15.3%; the ash from 11 to 29.3%; and the moisture from 2.3 to 8.4%. By varying the amounts of fish meals in the rations it was possible to adjust the protein content to 8% but, because of the great variations in fat content, the rations could not be adjusted on an isocaloric basis; also, because of the great variations in ash, the total mineral content of the rations

TABLE 1

Proximate chemical analyses of fish meals

FISH MEALS	PROTEIN	FAT	ASH	MOISTURE
	%	%	%	%
Alaska herring meal	69.7	8.4	11.0	6.0
Herring meal with fish solubles	52.1	15.3	15.4	8.4
Sardine meal	58.4	3.3	21.8	2.3
Menhaden fish meal	63.4	15.0	15.9	3.3
Red fish meal	57.1	8.1	25.3	6.0
Anchovies meal	50.3	3.3	26.1	6.0
Crab meal	35.0	2.1	29.3	7.0

was also different. However, it is evident from table 2 that the high mineral content of Red fish meal did not interfere with its excellent protein utilization; therefore, the lower net utilization of protein in the crab meal is not due to its high mineral content but rather to its low digestibility. The large standard deviations in the biological value and digestibility of crab meal are due to individual differences in nitrogen retention and digestibility. The standard deviations for digestibilities for Red fish meal and the herring meals seem high, but they are not any higher than that of dried non-fat milk solids. The least standard deviations for biological values and digestibilities were found for the proteins in dried whole eggs.

TABLE 2

The relative biological values of the proteins of various fish meals, dried whole eggs, and dried non-fat milk solids fed at an 8% protein level. Daily food intake, 8 gm. Daily nitrogen intake, 102.4 mg

SOURCE OF PROTEIN	BODY WEIGHT	FECAL N [1]	MET.[2] N IN FECES	FOOD N IN FECES	ABSORBED N	N IN URINE	MET. N IN URINE	FOOD N IN URINE	FOOD N RETAINED	TRUE DIGESTIBILITY	BIOLOGICAL VALUE [3]	NET UTILIZATION [4]
	gm	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	%	%		
Sardine meal	72.5	14.9	12.7	2.2	100.2	33.3	19.7	13.0	86.6	97.8	86.4	84.5
Menhaden fish meal	66.5	21.1	10.1	11.0	91.4	33.5	21.5	12.0	79.4	89.2	86.9	77.5
Red fish meal	67.3	23.7	10.2	13.5	88.9	33.8	22.5	11.3	77.6	86.8	87.3	75.8
Alaska herring meal	76.7	20.8	7.7	13.1	89.3	36.9	18.8	18.1	71.2	87.2	79.7	69.5
Herring meal with fish solubles	68.6	20.6	10.0	10.6	91.8	38.7	22.4	16.3	75.5	89.6	82.2	73.6
Anchovies meal	66.6	35.0	11.0	24.0	78.4	35.1	21.5	13.6	64.8	76.6	82.6	63.1
Crab meal	65.9	40.7	10.7	30.0	72.4	32.4	24.4	8.0	64.2	70.7	85.9	60.2
Dried non-fat milk solids	68.9	21.0	10.4	10.6	91.8	30.3	23.1	7.2	84.6	89.6	92.2	82.6
Dried whole eggs	78.8	13.3	11.2	2.1	100.3	25.5	22.0	3.5	96.8	97.9	96.5	94.5

[1] N signifies Nitrogen.
[2] Met. signifies Metabolic.
[3] Per cent of absorbed nitrogen retained in the body.
[4] The value for the true coefficient of digestibility multiplied by the biological value divided by 100.

It is apparent from table 2 that the proteins of dried whole eggs rank first in efficiency, followed by those of dried non-fat milk solids. However, all of the fish meals have high biological values. The lower net protein utilization of crab meal and anchovies is due to their low true digestibilities. When the net utilization of the proteins in dried whole eggs is taken as 100, those of dried non-fat milk solids are 88.4, and the various fish meals are calculated as follows: sardine meal, 89.4; Menhaden fish meal, 82.0; Red fish meal, 80.2; Alaska herring meal, 73.6; herring meal with fish solubles, 77.9; anchovies meal, 66.7; and crab meal, 63.7.

SUMMARY

A study was made of the biological values of various fish meals by the nitrogen retention method. All of the fish meals were found to have high biological values. However, because of low digestibilities, the net utilization of the proteins in crab meal and in anchovies meal was lower. The biological values were as follows: sardine meal, 86.4; Menhaden fish meal, 86.9; Red fish meal, 87.3; Alaska herring meal, 79.7; herring meal with fish solubles, 82.2; anchovies meal, 82.6; and crab meal, 85.9. When the net protein utilization of dried whole eggs is taken as 100, the various fish meals have the following values; sardine meal, 89.4; Menhaden fish meal, 82.0; Red fish meal, 80.2; Alaska herring meal, 73.6; herring meal with fish solubles, 77.9; anchovies meal, 66.7; and crab meal, 63.7.

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Nominations are solicited for the 1953 Award and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the Jury of Award may recommend that it will be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor

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Chairman, Nominating Committee:

DR. LEO T. SAMUELS
Department of Biological Chemistry
University of Utah Medical School
Salt Lake City, Utah

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Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1953 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1953.

Chairman, Nominating Committee:

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